Some effects of furazolidone in poultry

by

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Declaration

In compliance with the Edinburgh University Regulation 2.4.15, I, the undersigned, hereby declare that this thesis has been composed by myself and that the material in it is my own. It has not been submitted in any form for another degree or professional qualification.

In compliance with Regulation 2.4.11, reprints of one paper and two communications to the British Pharmacological Society describing some of the materials in this thesis are enclosed as an appendix.

B. H. Ali

Abstract

Treatment of chickens, ducklings and turkey poults with furazolidone (0.04% w/w, in the feed) for 10 days inhibited monoamine oxidase (MAO) activities in the alimentary tract, heart and brain. Only in the duckling was an inhibition of MAO activity found in the liver.

In the chicken and duckling, furazolidone (200 mg/kg) by crop tube inhibited MAO activities in the duodenal mucosa, liver, heart and brain, whereas the same dose of the drug given intramuscularly to the chicken was ineffective. Furazolidone by crop tube was about equipotent as an MAO inhibitor in chickens, ducklings and turkey poults. Pretreatment of chickens and ducklings with neomycin to suppress the alimentary microflora reduced the effect of furazolidone on MAO activity, suggesting that the microflora transformed the drug to an active metabolite which subsequently inhibited MAO activities in other organs.

Furazolidone (0.04% w/w, 10 days) increased the amount of 5-hydroxytryptamine (5-HT) in the brain of chickens and ducklings, and potentiated the vasopressor action of tyramine in chickens. The amounts of noradrenaline and adrenaline in the brain were unaffected by the treatment, as were the actions of noradrenaline, 5-HT, histamine and tryptamine on blood pressure.

In poultry, furazolidone (0.04% w/W, 10 days) produced an anorexia and an increase in the concentrations of pyruvate and lactate in blood. The activation

of transketolase by thiamin pyrophosphate was greater in haemolysates from furazolidone-treated chickens than in control preparations. These observations suggested a deficiency of thiamin in the furazolidone-treated birds. However, intramuscular administration of thiamin HCl (84 µg/kg, twice daily for 10 days) to the chicken did not prevent the development of signs characteristic of its deficiency.

In chickens given furazolidone (0.04% w/w, 10 days), the weights of the adrenal and thyroid glands (as % of body weight) and the oxygen consumption of hologenized heart were all increased.

Furazolidone (0.04% w/w, 10 days) did not produce an anaemia or affect the arterial blood pressure in the conscious chickens, and the activities of plasma aspartate transaminase and hepatic aminopyrine demethylase were unaffected. Ducklings survived when furazolidone was given in the feed at a concentration of 0.04% w/w for 10 days, or by crop tube at a dose of 200 mg/kg.

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RESULTS AND DISCUSSION

Summary

1. The addition of furazolidone to the feed at a concentration of 0.04% w/w for 10 days inhibited monoamine oxidase (MAO) activities in the alimentary tract, heart and brain of chickens, ducklings and turkey poults. In the chicken, the MAO inhibition was most marked in the rectal caeca and least in the liver, where no inhibition was found. Only in the duckling was an inhibition of the enzyme activity found in the liver.

2. Furazolidone (200 mg/kg) administered to chickens and ducklings by crop tube inhibited MAO activities in duodenal mucosa, liver, heart and brain. The same dose of furazolidone injected intramuscularly did not inhibit MAO activity in the chicken. Furazolidone (by crop tube) was about equipotent as an inhibitor of MAO activity in the chicken, duckling and turkey poult.

3. Pretreatment of chickens and ducklings with neomycin by crop tube to suppress the alimentary flora reduced the effect of furazolidone on MAO activity, suggesting that the drug was transformed by the alimentary flora to an active metabolite which subsequently inhibited MAO activity in other organs. However, pretreatment of chickens with intramuscular neomycin did not antagonize the inhibition of MAO activity produced by furazolidone (200 mg/kg, crop tube).

4. Furazolidone (0.04% w/w, 10 days), probably in

consequence of the MAO inhibition, increased the amount of 5-hydroxytryptamine (5-HT) in the brain of chickens and ducklings and potentiated the vasopressor action of tyramine in the chicken. The amounts of adrenaline and noradrenaline in the brain were unaffected by the treatment, as were the actions of noradrenaline, histamine, 5-HT and tryptamine on the blood pressure.

5. Furazolidone (0.04% w/w, 10 days) reduced the feed intake and growth of chickens, ducklings and turkey poults. The growth of chickens fed ad libitum with furazolidone (0.04% w/w) was similar to that of birds pair-fed with unmedicated diet, indicating that anorexia was mainly responsible for the reduction in growth in the drug-treated birds. Methergoline (an antagonist of central actions of 5-HT) did not affect the anorexia in the furazolidone-treated chickens, suggesting that it might not be an outcome of the increase in brain 5-HT.

6. Treatment of chickens with furazolidone (0.04% w/w, 10 days) increased the concentrations of pyruvate and lactate in blood, and the activation of transketolase by thiamin pyrophosphate in haemolysates. These signs together with the anorexia suggested a borderline deficiency of thiamin. However, the administration of thaimin HCl at a dose above the requirement of the birds (84 µg/kg, i.m. twice daily for 10 days) did not reverse the signs of its possible deficiency. It seems that

furazolidone might have interfered with the utilization of the vitamin.

7. In the chicken, the weights of the adrenal and thyroid glands (as % of body weight) were selectively increased following administration of furazolidone (0.04% w/w, 10 days). The increase in the weight of the adrenal glands most probably represents hypertrophy of the cortex, as the amount of catecholamines in the glands was unaffected by furazolidone.

8. The oxygen consumption of homogenized heart was increased when the chickens were pretreated with furazolidone (0.04% w/w, 10 days), whereas the utilization of oxygen by other tissues was unaffected by the treatment.

9. Furazolidone (0.04% w/w, 10 days) did not produce anaemia or affect the arterial blood pressure in conscious chickens.

10. The activities of hepatic aminopyrine demethylase and plasma aspartate transaminase were unaffected by furazolidone (0.04% w/w, 10 days) in the chicken.

11. Although furazolidone is considered highly toxic in the duck, in the present experiments ducklings survived when it was given in the feed at a concentration of 0.04% w/w for 10 days or by crop tube at a dose of 200 mg/kg. It is suggested that the husbandry of the duck affects its susceptibility to furazolidone.

Introduction

In a world with an increasing population and consequently ever-increasing demand for animal protein, poultry production occupies an important place. The use of drugs as additives to poultry feed has significantly contributed to the rapid developments in the quality and quantity of poultry products, by virtue of increasing feed conversion and reducing mortality of growing birds. The addition of furazolidone to poultry feed is recommended for the therapy and prevention of certain protozoal and bacterial diseases.

Furazolidone; history and properties

Furazolidone [3-(5-nitrofurfurylidene amino)-2-oxazolidone] (fig.1) is a member of a group of drugs known as the nitrofurans which are synthetic organic compounds obtained initially from furfural, an agricultural by-product from corn and oat husks. The activity of these compounds as antimicrobial agents was first studied by Dodd & Stillman (1944), who noted that a nitro-group in the 5-position of 2-substituted furans conferred antibacterial activity on the compounds. The addition of various side chains to the 2-position of the furan ring largely governs the chemical properties (solubility, melting point, etc.) and clinical usefulness of the nitrofurans. Although more than 3500 nitrofurans have been synthesized, only a few have found a place in chemotherapy. The antimicrobial properties of

furazolidone was first reported by Yurchenco, Yurchenco & Pieopli (1953). Thereafter, several reports on the antimicrobial properties of this drug in different animal species appeared in the literature.

The physical properties of furazolidone include a molecular weight of 225.2 and a melting point of 255°C. The drug is an odourless yellow crystalline powder. It is initially tasteless but a bitter aftertaste follows. Furazolidone is only sparingly soluble in water, ethanol and organic solvents (the aqueous solubility at pH 6.0 is 40 mg/ml at 25°C). In dilute solution, the drug is unstable to light (Paul & Paul, 1964).

There is little information on the pharmacology of furazolidone in poultry although the drug is commonly used in the prevention and treatment of many diseases. The following review attempts to survey the work which has been reported on this drug in man and animals, with special emphasis on avian studies.

Methods for the estimation of furazolidone and their validity

Buzard, Vrablic & Paul (1956) have reported a method for the determination of furazolidone in plasma which depends on the formation and colorimetric estimation of 5-nitro-2-furaldehyde phenylhydrazone (NFP). Using this method, 'furazolidone' was detected in rat plasma at levels of 3.3, 3.6, 1.3 and 1.6 µg/mℓ at 4, 8, 16 and 24 h, respectively, following a single oral dose of 500 mg/Kg. This method had to be modified because it was found

difficult to apply in highly pigmented tissues like liver due to non-specific colorimetric interference. Chromatographic columns were used to separate the phenylhydrazone from non-specific liver chromogen (Herrett& Buzard, 1960). This method allowed the detection of the 'drug' in concentrations as low as 0.5 µg/g tissue and permitted the quantitative determination of 'furazolidone' at 1-5 µg/g tissue, and this has been used for the determination of 'furazolidone' in chicken tissues.

A method of analysis (Beckman, 1958) that makes use of the colours produced when furazolidone is treated with strong alkalis has been described, but is difficult to apply due to the instability and lack of reproducibility of the colours.

A bioassay of furazolidone made use of the inhibition of growth of drug-susceptible organisms, such as Escherichia coli or Staphylococcus aureus (Paul & Paul, 1964).

Hollifield & Conklin (1968) reported a method for the determination of furazolidone which depends on the fact that when the drug is mixed with p-diisobutyl-cresoxyethyoxyethyl dimethylbenzyl ammonium hydroxide (Hyamine hydroxide), it exhibits an absorption maximum at 600 nm. Using this method, they could not detect furazolidone in dog or human urine following oral administration of 5 mg furazolidone/Kg. Recoveries of the drug (2-50µg/mℓ) added to dog or human urine were 99 and 102%, respectively. For comparison, the urine samples were also analyzed for furazolidone by methods in which the drug

is converted to its phenylhydrazone (NFP) (Nakamura & Inoue, 1961; Buzard et al, 1956). The presence of materials convertible to NFP was confirmed in human and dog urine following furazolidone treatment. Hollifield & Conklin (1968) concluded that the (NFP) methods for detecting furazolidone are not specific, and often measure metabolite(s) of the drug rather than the drug itself.

Absorption, metabolism and excretion of furazolidone and its metabolites

Furazolidone is only very sparingly soluble in water, alcohol, or organic solvents. It is not surprising, then, that it is poorly absorbed from the gastrointestinal tract, mucosal membranes, or through intact skin (Omer, 1978). The absorption of a nitrofuran drug depends in part on the solubility of the compound in the contents of the alimentary tract, and on the rate of its degradation in the mucosae of the small intestine (Tatsumi, Ou, Yamaguchi & Yoshimura, 1973).

Studies with rat tissues in vitro (Rogers, Belloff, Paul, Yurchenco & Gever, 1956) have shown that all tissues, with the exception of blood, rapidly degrade furazolidone. When furazolidone was given to colostomized chickens at an oral dose of 30 mg/Kg, it was rapidly and extensively metabolized and the metabolites excreted in urine (Craine & Ray, 1972). In these experiments faecal excretion of furazolidone metabolites was not examined.

Following an oral administration of furazolidone

(5 mg/Kg) to man and dog, the drug could not be detected in urine (Hollifield & Conklin, 1968). However, chromatography of these urine samples revealed the presence of 2 metabolites. One matabolite was yellow and the other was orange in appearance under U.V. light. The two metabolites were present in different relative amounts in man and dog urine and they demonstrated little inhibitory activity against Escherichia coli, against which furazolidone exhibits significant inhibitory activity. Tennent & Ray (1971) found that in swine, orally administered furazolidone is excreted in the form of a large number of metabolic end products. When furazolidone labelled with C^{14} was given orally to a pig, 71% and 19% of the labelled dose was recovered (as metabolites) in urine and faeces, respectively.

Drug residues in furazolidone-treated birds

Data on drug residues in furazolidone-treated birds were obtained using the (NFP) method, which is of dubious validity as it does not distinguish between the parent drug and its metabolites. Thus it seems that the 'drug' levels reported represent furazolidone plus certain metabolites.

No evidence of accumulation of 'furazolidone' was obtained in eggs or tissues of hens receiving furazolidone at a dose of 0.05% w/w for 28 days (Belloff, Buzard & Roberts, 1953). However, other workers (Kreig & Löliger, 1973) using a similar analytical method, reported that residues of 'furazolidone' (2.0-2.5 µg/mℓ of blood and 0.06-0.16 µg/g tissue) were present in chickens given the

drug at single oral doses ranging from 20-200 mg/Kg, or in the feed at concentrations of 0.04% w/w for 7-14 days. In all cases blood and tissues were free from residues 2 days after withdrawal of the drug. It was also reported (Krieg, 1972) that hens receiving furazolidone at an oral dose of 20-100 mg/bird for 8 days laid eggs containing 15-70 µg of the 'drug' and that levels in the eggs declined to nil 5-6 days after withdrawal of the drug.

Antimicrobial properties of furazolidone

Furazolidone is endowed with antimicrobial activity against a wide variety of microorganisms. In vitro, the drug was shown to be active against the gram-ve organisms, Escherichia coli, Klebsiella, Salmonella, Shigella, Enterobacter, Bacteriodes and Campylobacter cholera. A lower order of activity was found with Proteus. It is also active against the gram+ve bacteria Staphylococcus and Streptococcus, and against the protozoa Trichomonad and Giardia (Chamberlain, 1976). In man, the drug has found many applications in the tropics as an antimicrobial agent in the alimentary tract and vagina (Ponce de Leon, 1957; Schneierson & Bryer, 1959; Limaye, 1980). The drug is also given orally against a wide range of gastrointestinal diseases of poultry, pigs, horses and foxes (Omer, 1978). A brief summary of the therapeutic and prophylactic uses of furazolidone in poultry is given in table (1).

Toxicity of furazolidone

The following is an account of some of the toxic

effects produced by furazolidone, which have been reported in some mammalian and avian species.

Toxicity in mammals In rats, furazolidone at an oral dose of 50 mg/Kg inhibited 81% of the activity of monoamine oxidase (MAO) in the liver (Stern, Hollifield, Wilk & Buzard, 1967), and at a higher dose (500 mg/Kg) it produced 95% inhibition of MAO activity in both liver and brain of rats (Palm, Magnus, Grobecker & Jonsson, 1967). In man, oral administration of furazolidone (10 mg/Kg for 10 days) caused anorexia, restlessness, disorder of sleep and orthostatic collapse (Palm & Magnus, 1968), and higher doses (80-140 mg/Kg for 15 days) inhibited 85% of MAO activity in jejunal mucosa, augmented the pressor actions of tyramine and amphetamine and increased the urinary excretion of tryptamine (Pettinger, Soyangco & Oates, 1968).

The calf was shown to be highly susceptible to furazolidone toxicity. The nature of furazolidone toxicity in this species is different from that in rat and man. Doses of 4-9 mg/Kg for 40 days (Hoffmann & Frezer, 1974) or 8.5 mg/Kg for 7 weeks (Hoyashi, Yamane & Sakai, 1976) caused haemorrhagic diathesis, ascites, changes in haemogram and lesions in bone marrow, kidney, liver and lung. Relatively high doses of the drug (20-30 mg/Kg) caused tonic spasms and excitation (Hoffmann, Reinacher & Dirkenson, 1977). In calves and pigs, furazolidone at doses of 5 or 10 mg/Kg influenced the extrasecretory function of the pancreas (Khomenko, 1979). It decreased the volume of the pancreatic fluid of both species

and increased the proteolytic activity of that of pigs.

A nervous syndrome was observed in a herd of sows which had been given furazolidone in the feed at a concentration of 0.63% w/w 7-10 days post-weaning (Borland, 1979). The toxic signs observed were ataxia, crouching, 'goose-stepping', leaping high in the air, and lying on the ground on their sides. Affected animals recovered within a few days on withdrawal of the drug.

It seems that the dog and G. pig are not very susceptible to furazolidone toxicity. In G. pigs, high doses ranging from 735 - 2,025 mg/Kg for 7 days reduced the body weight by 16% and caused some histopathological changes in testes, adrenals and stomach (Koeda, Saskai, Kohanwa & Konnon, 1976). A dose of 7.5 mg/Kg for 6 months did not produce any clinical signs in the dog, although some histopathological changes in the central nervous system and testes were noted, and a single oral dose of 200 mg/Kg resulted in emesis in half the animals tested (Rogers et al, 1956).

Furazolidone has been reported to be carcinogenic to rats and mice when it is given continuously over a year or for life (Anonymous, 1976 a, b). Initial studies showed that, following furazolidone treatment (0.15% w/w for one year) breast tumors were induced in female rats. Lifetime studies were also conducted in two strains of rat and these showed that furazolidone given in the feed (at

several doses up to 0.1% w/w) caused a significant increase in the incidence of breast tumors (particularly adenocarcinomas) in the female, and that the incidence was dose-related. In mice, however, furazolidone (up to 0.03% w/w in the feed for life) induced bronchial adenocarcinoma in both sexes.

Toxicity in birds

The recommended therapeutic dose of furazolidone in chickens and turkeys is 0.04% w/w in the feed for 10 days, and in ducks, 0.01% w/w in the feed for 7 days (Veterinary Data Sheet, 1980). The drug at doses equivalent to or slightly above the therapeutic level has been reported to cause toxic signs in birds. These are summarized below:-

1. Toxicity in chickens

Radchuck (1965) reported signs of toxicity in chicks and hens 4-6 h after oral administration of 300 mg/Kg. The birds became inappetent, drowsy and unaware of the surrounding environment. These signs were followed by paralysis and death. The LD_{50} of the drug was found to be 380 mg/Kg, and 500 mg/Kg was lethal to all the birds studied. However, Harwood & Stunz (1954) estimated that the LD_{50} of furazolidone in the chicken was 500 mg/Kg.

The effect of furazolidone on chicken growth was first studied in 1953, when it was shown that feeding furazolidone at a low level (0.006% w/w for 10 weeks) did not cause a beneficial effect on growth of chickens kept in a clean

environment (Jacobs, Elam, Andreson, Gee, Fowler & Couch, 1953). Subsequently, this observation was confirmed by other workers (Harwood & Stunz, 1954; Cooper & Skulski, 1956). It has also been shown (Harwood & Stunz, 1954; McDonald & Beilharz, 1961; Ehlhardt, Beuving & Haye, 1975; Jensen, Chang & Washburn, 1975) that feeding furazolidone at levels of 0.033% w/w for 4 weeks or 0.04% w/w for variable periods caused a significant reduction in growth. The cause of this has been attributed to impaired digestion, absorption or metabolism in the birds. Sauer, Jensen & Schutze (1969) reported a significant reduction in the weight of eggs from chickens fed furazolidone at a concentration of 0.003 - 0.011% w/w for 4-6 weeks. More recently, Czarnecki & Sujarit (1979) showed that furazolidone (60 µg or more) injected into the chicken egg at 40 and 72 h of incubation, significantly reduced the growth and increased the glycogen level in the chicken embryc.

Furazolidone treatment (0.055% w/w in the feed for 4 weeks) caused ascites and a 30% mortality (Harwood, Stunz & Wolfgang, 1958), and at a concentration of 0.04% w/w for 6 weeks, the drug caused a cardiomyopathy associated with cardiac dilation and ascites (Feron & Van Stratum, 1966). A small increase (4.4%) in the heart weight was reported by Francis & Schaffner (1956) following furazolidone feeding (0.017% w/w for 3 weeks). Other workers (Jensen et al, 1975), however, were unable to

confirm these effects on the heart when furazolidone was fed to chickens at a concentration of 0.07% w/w for 4 weeks.

Nervous signs (unspecified) were observed in chicks fed furazolidone at a concentration of 0.2% w/w for 1-13 days (Smith, 1955, cited by Cooper & Skulski, 1956.)

A small but significant reduction in packed cell volume and haemoglobin content was reported by Ehlhardt et al (1975) when furazolidone was fed to chickens at a level of 0.04% w/w for 3 weeks. No anaemia was found, however, in chicks fed furazolidone (0.01-0.07% w/w for 4 weeks by Jensen, et al (1975).

Reports on the effect of furazolidone on the weight of the thyroid gland are at variance. Mellen & Waller (1954) noted a 24% increase in the weight of the gland when furazolidone was fed at a level of 0.011% w/w for 8 weeks. However, Francis & Schaffner (1956), reported a 14% reduction in the thyroid weight following furazolidone treatment (0.022% w/w for 4 weeks).

Cooper & Skulski (1955, 1956) fed furazolidone at concentrations of 0.005-0.08% w/w for variable periods up to 27 weeks, and noted a reversible retardation of spermatogenesis and a significant reduction in the testes weights. The drug did not seem to have an adverse effect on the fertility of the birds or the hatchability of the eggs.

2. Toxicity in turkeys

Cardiomyopathy and cardiac dilation seem to be the major toxic signs of furazolidone in turkeys. Several workers (Jankus, Noren & Staley, 1972; Czarnecki, Jankus & Hultgren, 1974; Czarnecki, Reneau & Jankus, 1975; Jensen et al, 1975) have found that cardiomyopathy (right and left ventricular dilation), ascites, growth depression, and death occurred when turkey poults were fed furazolidone at concentrations ranging from 0.03-0.07% w/w for 3 weeks or more.

Furazolidone has been shown to produce some biochemical changes in turkeys. Staley, Noren, Bandt & Sharp (1978) reported that feeding furazolidone (0.05% w/w for 4 weeks) caused a disordered hepatic metabolism, as judged by a lowering of total plasma proteins, an increased activity of aspartate transaminase in serum and an increase in the level of glycogen in the liver. These effects have been confirmed by Simpson, Rollinghoff, Preisig & Fisher (1979), who also showed that furazolidone treatment (0.05% w/w for 24 days, followed by 0.07% w/w for 42 days) resulted in hypotension and a decrease in the proteins and trypsin inhibitory factor in the plasma.

3. Toxicity in ducks

Acute toxicity has been reported in mallard ducklings, aged 4-6 weeks, following administration of furazolidone (100 mg/bird) on 2 successive days (Lucas, 1956).

On the first day of treatment, no adverse effects were noted. However, within 15-30 minutes of the second dose, the birds would jump up, run blindly into fences or other objects, whirl around in circles, fall down, and after lying motionless for a time, would eventually arise and act normal. Dougherty (1956) reported toxic signs (unspecified) in ducklings fed furazolidone at a concentration of 0.022% w/w for 10 days. It has also been reported that treatment of ducklings with furazolidone (0.062% w/w - 0.092% w/w for 18 days) resulted in depression or excitation, mobility disturbance and death (Kriz, Branda & Svestka, 1968).

4. Toxicity in pigeons

Pigeons seem to tolerate high doses of furazolidone (Timchenko, 1968). Toxic signs appeared 3 hours after an oral dose of 1 g/Kg and continued for 24 hours. These signs were nervous in nature and included depression, incoordination of movement and drowsiness. One bird out of 6 died when 1.5 g/Kg was given orally. In the rest there were nervous signs, anorexia and diarrhoea. The LD_{50} of furazolidone in pigeons was found to be 2375 mg/Kg.

Aims of the present study

Despite the widespread use of furazolidone in poultry, its pharmacology seems to have received insufficient attention in these species. This warranted a pharmacological study of furazolidone in poultry, re-examining some of the effects described before and searching for further pharmacological and biochemical actions of the drug.

Materials

Animals

Chickens (T-line) and turkey poults (Ross Super-Midi Breeder hybrid) were obtained from the Poultry Research Centre, Roslin. Duck eggs (Alesbury, Khaki-Campell and Indian Runner) were bought from farms in Dalkeith and Saline and hatched in an incubator in the Department of Veterinary Pathology.

The birds were housed in the Wellcome Animal Research Unit in cages containing a deep litter of white wood shavings. Each cage (110 x 70 x 60 cm) accommodated 3-4 birds. In some experiments, cages were divided with a wire mesh and one bird accommodated in each half. The individually-housed birds had visual and auditory contact with each other. Chickens were kept at a temperature of 21°-24°C, and chicks, ducklings and turkey poults at 24°-28°C, with 50-60% relative humidity and 10h of artificial light daily.

The chickens were fed a layer mash supplied by McGregor & Co (Leith), Quayside Mills, Leith, Edinburgh; and chicks, ducklings and turkey poults were given the appropriate 'starter' ration supplied by the Poultry Research Centre (Roslin). All the diets were free from growth-promoting and anticoccidial agents, and were usually available to the birds *ad libitum*.

In one experiment chickens (7½ weeks old) were housed singly and fed ad libitum with unmedicated feed or feed containing furazolidone (0.4% w/w) for 10 days. The growth and feed intake of the birds were recorded daily, and the values for the feed intake used subsequently for a "paired-feeding" experiment. In this experiment the chickens were provided daily with a measured amount of unmedicated feed, which was equivalent to that consumed by the control or the furazolidone-treated birds.

Drugs and Chemicals

The drugs and chemicals used were :- acacia (M & B, Laboratory Chemical), acetylacetone, redistilled (Koch Light Laboratory, Laboratory Reagent), (±) adrenaline hydrogen tartarate (B.D.H., Biochemical grade), amberlite resin, CG_{50}, hydrogen form, mesh size 100-200 (B.D.H., Chromatographic grade), 4-aminophenazone (B.D.H., Laboratory Reagent), aminopyrine (4-dimethylamino-antipyrine, 97% w/w) (Ralph N. Emanual), ammonium acetate (B.D.H., Analytical Reagent), ammonia solution (about 35% NH_3) (B.D.H., Analytical Reagent), anthrone (Sigma), ascorbic acid (B.D.H., Analytical Reagent), L-aspartic acid (Sigma), barium hydroxide (B.D.H., Analytical Reagent), cholestrol (Sigma), cyanomethaemoglobin (B.D.H., Clinical Reagent), α-α'-dipyridyl (Sigma), dopamine (3-hydroxytyramine) (Sigma), ferric chloride, hexahydrate (B.D.H., Analytical Reagent),

formaldehyde, 40% w/v solution (B.D.H., Analytical Reagent), furazolidone (micronized preparation, particle size 5 μm) (Eaton Laboratories), a premix containing furazolidone, 4% w/w (Neftin) (Eaton Laboratories), D-glucose (B.D.H., Analytical Reagent), glucose oxidase (Sigma), D-glucose-6-phosphate monosodium salt (Sigma), glycine (B.D.H., Biochemical grade), Halothane (Fluothane) (I.C.I.), heparin sodium (Sigma), histamine dihydrobromide (Sigma), hydrazine hydrate (B.D.H., Analytical Reagent), 5-hydroxytryptamine creatinine sulphate (B.D.H., Biochemical grade), 4-hydroxyquinoline (Sigma), hydrochloric acid (B.D.H., Micro Analytical Reagent), α-ketoglutaric acid (2-oxoglutaric acid) (Sigma), kynuramine dihydrobromide (Sigma), lactate dehydrogenase, III (Sigma), L (+) lactic acid (Sigma), magnesium chloride, hexahydrate (B.D.H., Analytical Reagent), magnesium sulphate, heptahydrate (B.D.H., Analytical Reagent), malate dehydrogenase (Sigma), metaphosphoric acid (B.D.H., Laboratory Reagent), methergoline (Farmitalia), neomycin sulphate, 70% w/w, soluble powder, veterinary (Squibb & Sons, Ltd), nicotinamide (nicotinic acid amide) (Sigma), nicotinamide adenine dinucleotide, reduced form (NADH), grade III (Sigma), nicotinamide adenine dinucleotide phosphate (NADP), monosodium salt (Sigma), (-) noradrenaline hydrogen tartarate (Sigma), orthophosphoric acid, 80% (B.D.H., Analytical Reagent), Orcinol, crystalline (Sigma), perchloric acid, 71-73% w/w (B.D.H., Analytical Reagent), peroxidase, salt-free

powder, type I, from horseradish (Sigma), phenobarbitone sodium (B.D.H., Laboratory Reagent), phenol (B.D.H., Analytical Reagent), potassium carbonate (B.D.H., Analytical Reagent), potassium chloride (B.D.H., Analytical Reagent), potassium dichromate (B.D.H., Analytical Reagent), potassium dihydrogen orthophosphate (B.D.H., Analytical Reagent), potassium ferricyanide (M & B, Laboratory Reagent), potassium hydroxide (B.D.H., Analytical Reagent), potassium phosphate (B.D.H., Laboratory Reagent), ribose (Sigma), ribose-5-phosphate monobarium salt (Sigma), semicarbazide HCl (B.D.H., Analytical Reagent), sodium bicarbonate (B.D.H., Analytical Reagent), sodium chloride (B.D.H., Analytical Reagent), di-sodium hydrogen orthophosphate dihydrate (B.D.H., Laboratory Reagent), sodium hydroxide (B.D.H., Analytical Reagent), sodium pyruvate (Sigma), sodium sulphate, decahydrate (B.D.H., Analytical Reagent), sodium tungstate (B.D.H., Analytical Reagent), Sulphuric acid (B.D.H., Analytical Reagent), thiamin (Sigma), thiamin pyrophosphate (Sigma), thiourea (Griffin & George, Laboratory Reagent), trichloroacetic acid (B.D.H., Analytical Reagent), tris (hydroxymethyl) amino-methane (B.D.H., Analytical Reagent), tryptamine HCl (Sigma), tyramine HCl (Sigma) and zinc sulphate (B.D.H., Analytical Reagent).

Buffers and Solutions

0.5M phosphate buffer solution, pH 7.4, was made by mixing 0.5 M $Na_2HPO_4.2\ H_2O$ (80.3 ml) and 0.5 M KH_2PO_4 (19.7 ml). 0.067 M phosphate buffer solution, pH 7.4, was obtained by mixing 1 N HCl (13.3 ml) and 0.25 M Na_2HPO_4 (267 ml) and making up to 1 litre with distilled water. 1 M phosphate buffer, pH 7.4, was made by dissolving KH_2PO_4 (136 g) and NaOH (33 g) in distilled water and the volume adjusted to 1 litre. 0.01 M phosphate buffer, pH 7.0, was prepared by mixing 0.2 M NaH_2PO_4 (3.9 ml) and 0.2 M Na_2HPO_4 (6.1 ml), and making up to 200 ml with distilled water. 0.75 M Tris buffer, pH 7.4, was made by dissolving Tris (hydroxymethyl) aminomethane (90.8 g) in 1 litre of distilled water. 1 M sodium acetate buffer, pH 6.5, was made by dissolving NaOH (40 g) in distilled water (900 ml) and glacial acetic acid (60 ml), and the volume made up to 1 litre with distilled water. 0.1 M phosphate buffer, pH 7.2, was obtained by mixing 0.1 M KH_2PO_4 (29.6 ml) and 0.1 M $Na_2HPO_4.2H_2O$ (70.4 ml). 0.5 M Glycine-0.4 M hydrazine buffer was made by dissolving glycine (37.5 g) and hydrazine hydrate (20 ml) in distilled water (300 ml), and adjusting to pH 9.0 with NaOH before making up the solution to 1 litre with distilled water. The saline-phosphate medium used in the estimation of transketolase activity was made by dissolving NaCl (2.81 g), KCl (9.26 g), K_2HPO_4 (2.73 g) and $MgSO_4.7H_2O$ (0.30 g) in distilled water and adjusting the pH to 7.4 with 1 N HCl. The volume was made up to

a litre with distilled water.

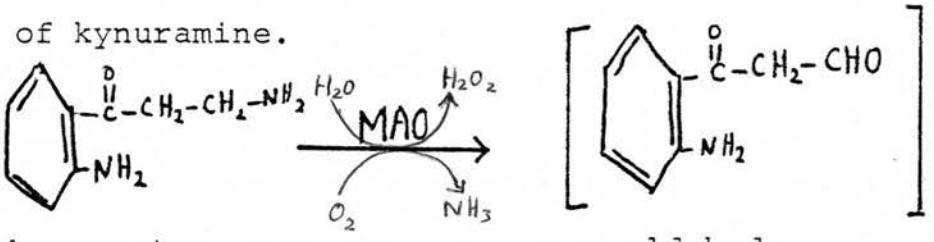
The pH of the buffer solutions was measured at a glass electrode (Albert Measurements Ltd, Model 40), or by using narrow range indicator papers (Whatman, B.D.H. Chemicals).

Mixing Furazolidone with the Food

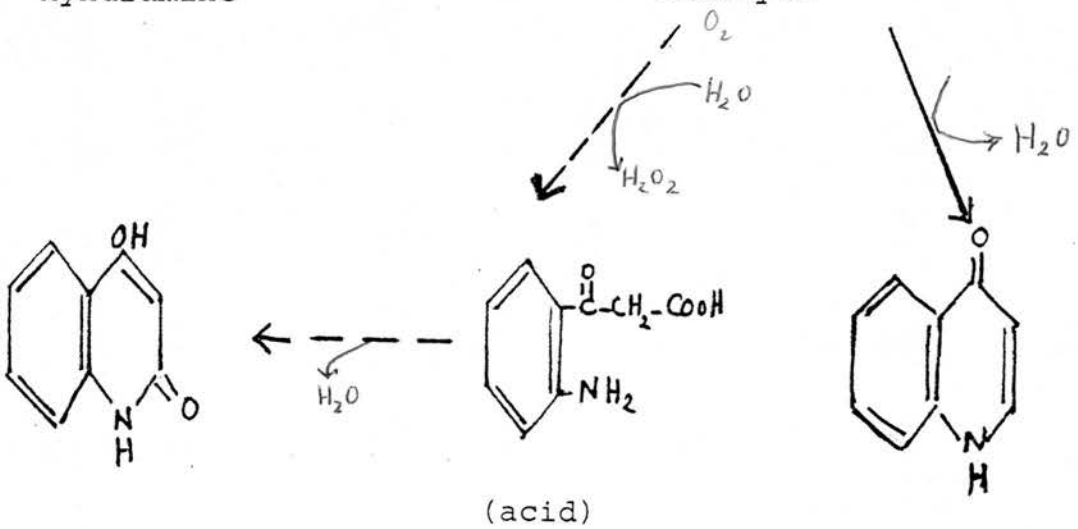
Furazolidone was fed to the birds at a concentration of 0.04% w/w for 10 days. A premix (Neftin) containing 4% w/w furazolidone was thoroughly mixed with the food (mash) using a pestle and a mortar. Enough food was mixed with the drug to last 5 days. Before the medicated food was given to the birds, it was remixed to ensure an even distribution of the drug in the food.

Assay of monoamine oxidase (MAO) activity

The method used for the estimation of MAO activity was that of Krajl (1965) which is based on monitoring fluorometrically the appearance of 4-hydroxyquinoline which arises from the spontaneous cyclization of the intermediate aldehyde formed by the oxidative deamination of kynuramine.



kynuramine aldehyde



(acid)

4-hydroxy-2(1 H)-quinoline 4-hydroxyquinoline

The intramolecular (non-enzymatic) condensation of the amino aldehyde to 4-hydroxyquinoline is faster than the further oxidation of the aldehyde to the acid or 4-hydroxy-2(1 H)-quinoline (Weissbach, Smith, Daly, Witkop & Udenfriend, 1960).

Preparation of tissues

The birds were killed by decapitation and the tissues rapidly removed and chilled on ice. The lumen of the duodenum was washed through with chilled saline before cutting it open and scraping away the mucosa with a glass slide. The heart was opened and blotted with filter paper to remove blood. The tissues were cut into small pieces and homogenized with 6 strokes in a teflon/ glass homogenizer in an ice bath. Homogenates (20% w/v) of each organ in 0.5 M phosphate buffer (pH 7.4) were prepared, and the appropriate dilutions made in the same buffer for the estimation of MAO activity.

Incubation medium

The incubation medium contained, 0.5 M phosphate buffer, pH 7.4 (0.5 ml), tissue homogenates (1 ml equivalent to 0.78 mg duodenal mucosa, 3.13 mg liver or brain or 12.5 mg heart), 0.02% w/v kynuramine dihydrobromide (0.5 ml) and distilled water to 3 ml.

An incubate without substrate served as the blank. The reaction mixtures were incubated at 37°C for 30 minutes under air and with shaking. The incubates were then deproteinized with 0.6 M perchloric acid (2 ml), as recommended by Century & Rupp (1968), followed by centrifugation at 900 g for 10 minutes at 5°C in a low-speed centrifuge (MSE MISTRAL 2L). Supernatant (1 ml) was mixed with IN NaOH (2 ml) and placed in a quartz cuvette. The solution was activated at 315 nm and the peak in the fluorescence scan at 380 measured in an Aminco-Bowman

spectrofluorimeter. The presence of substances affecting the fluorescence of 4-hydroxyquinoline was sought by mixing equal volumes of tissue blank and 4-hydroxyquinoline solution. In all cases the fluorescence of the mixture was half that of the undiluted solution of 4-hydroxyquinoline, demonstrating an absence of augmentation or quenching of fluorescence.

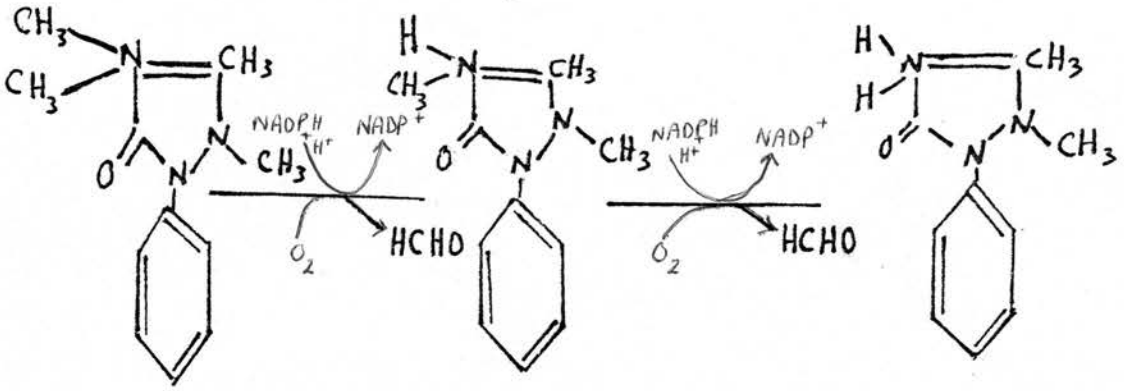
Internal standards of 4-hydroxyquinoline (0.25, 0.50, 1.0 and 2.0 µg/ml) were taken through the procedure described above, and a graph relating 4-hydroxyquinoline concentration with fluorescence intensity was constructed with every batch of tissue homogenates (fig.2). From this curve, fluorescence readings were converted to amounts (µg) of 4-hydroxyquinoline.

MAO activity (µ mol 4-hydroxyquinoline/g tissue/h) was obtained from the expression:-

$$\left[\frac{\text{4-hydroxyquinoline in medium} \times 2 \times 1000}{\text{Molecular wt. of 4-hydroxyquinoline} \times \text{wt. of tissue(mg)}}\right]$$

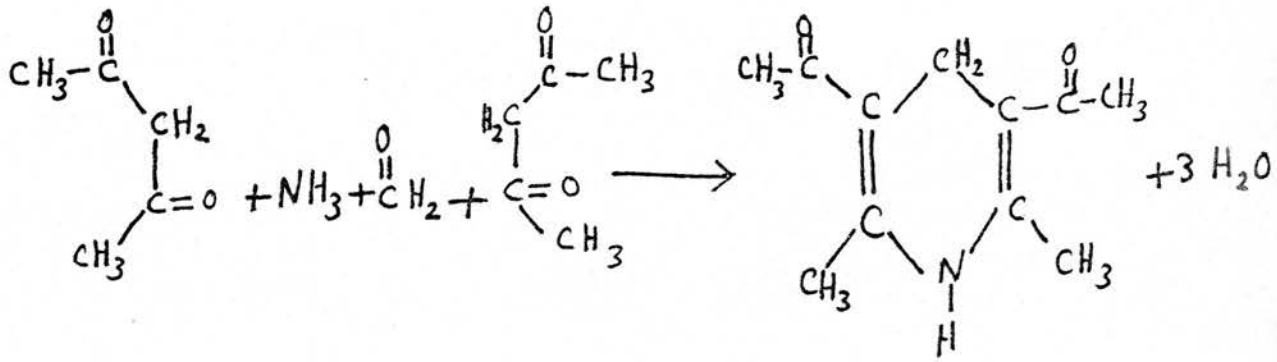
Measurement of aminopyrine demethylase activity in the liver

The activity of aminopyrine demethylase was measured by a method described by Mazel (1971), in which the production of formaldehyde from the demethylation of the substrate aminopyrine is measured spectrophotometrically.



aminopyrine — monomethyl-4 aminoantipyrine — 4-aminoantipyrine

The formaldehyde formed was trapped as the semicarbazone and measured by the colorimetric procedure of Nash based on the Hantzsch reaction (Nash, 1953).



2 acetylacetone + ammonia + formaldehyde

3,5-diacetyl-1,4-dihydrolutidine + 3 water

Preparation of tissues

Chickens (8 weeks old) were decapitated and pieces of liver excised, weighed and homogenized with chilled isotonic KCl (1.15% w/v) in a Teflon/glass homogenizer. The homogenates (25% w/v) were centrifuged at 10,000 g for 10 minutes at 5°C to remove nuclei and mitochondria. The microsome-rich supernatants were used for the enzyme assay.

Incubation procedure

A solution (1 ml) containing aminopyrine (13.88 mg/ml) and magnesium chloride (5.07 mg/ml) was pipetted into a 25 ml flask standing on ice, together with 3ml of a solution containing NADP (0.166 mg/ml), glucose 6-phosphate (0.94 mg/ml), nicotinamide (2.034 mg/ml) and semicarbazide HCl (2.23 mg/ml) in 0.5 M phosphate buffer, pH 7.4; and to these was added 2 ml of 10,000 g liver supernatant. Tissue blanks were prepared in which distilled water (1 ml) was substituted for the solution containing aminopyrine and $MgCl_2$. The reaction mixtures were incubated in a water bath at 37°C for 30 minutes with shaking. The reaction was stopped by the addition of 15% w/v zinc sulphate (2 ml), and the contents of the flasks were mixed and left 5 minutes before a saturated solution of barium hydroxide (2 ml) was added to each flask with mixing. After 5 minutes the contents of the flasks were transferred to centrifuge tubes and spun down at 900 g for 15 minutes

at 5°C. An aliquot of the supernatant (5 ml) was transferred to another flask which contained Nash Reagent (30% w/v ammonium acetate and 0.3% v/v acetylacetone) (2 ml). The contents of the flasks were mixed and incubated in the dark in a water bath for 1 hour at 37°C with shaking, to allow the development of yellow colour.

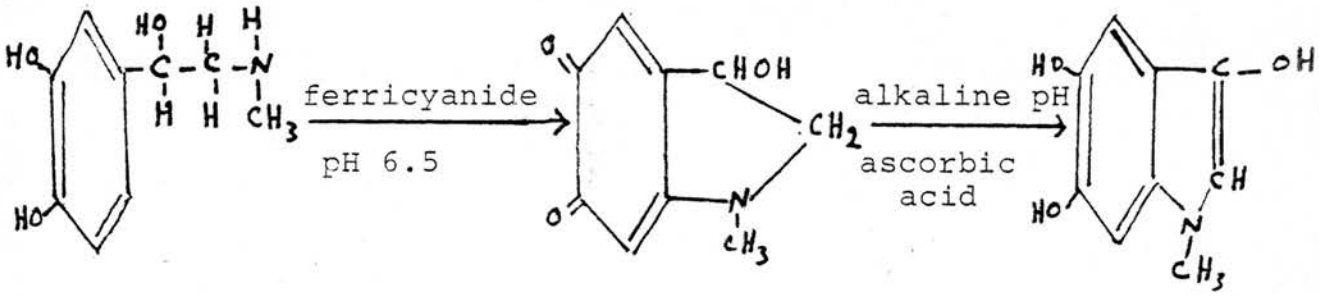
Standard solutions of formaldehyde (0.5, 1, 2 and 4 µg/ml) were prepared in each experiment (fig.3). An aliquot of each concentration of formaldehyde (5 ml) and a blank (distilled water) (5 ml) were mixed with Nash Reagent (2 ml) and incubated in the dark at 37°C for 1 hour in a water bath with shaking. The absorbence of the yellow colour was read in a spectrophotometer at a wavelength of 415 nm with the blank set at zero. The activity of aminopyrine demethylase in terms of formaldehyde produced (µmolHCHO/ml*/h) was obtained from the expression:-

$$\left[\frac{\text{quantity of HCHO produced by 5 ml of supernatant reacted with Nash Reagent (µg)} \times 2}{\text{molecular weight of formaldehyde}}\right]$$

* 10,000 g supernatant of liver homogenate (25% w/v).

Estimation of monoamines in organs of chickens and ducklings

The catecholamines adrenaline and noradrenaline were estimated in the brains and adrenals by the trihydroxyindole (THI) method of von Euler & Floding (1955) in which adrenaline and noradrenaline are oxidized in a buffered solution at a slightly acid pH to form their quinone derivatives, adrenochrome and noradrenochrome. In alkaline solution (NaOH), and in the presence of a reducing agent (ascorbic acid) to prevent oxidation, these chrome compounds rearrange to form the corresponding trihydroxyindoles adrenolutine and noradrenolutine.



adrenaline adrenochrome adrenolutine

The lutines fluoresce strongly under U.V. light, with characteristic activation and emission spectra. Since adrenaline will oxidize at a lower pH than noradrenaline, it is possible to estimate the fluorescence due to adrenaline in a mixture of the amines.

The estimation of monoamines involved the following

steps:-

1) Extraction of the amines from the tissues.

2) Purification so as to remove all contaminants.

3) Fluorimetric estimation of the catecholamines and 5-HT present.

Preparation of tissue extracts

Chickens aged 8 weeks or chicks and ducklings aged 12-17 days were decapitated and the whole brain or adrenals rapidly dissected out and placed on glass plates cooled on ice. The tissues were homogenized in ice-cold 0.1 M HCl (2 ml/g) containing ascorbic acid (5 mg/ml) by six strokes in a Teflon homogenizer, and diluted with distilled water to a volume of 11 ml (brain) or 50 ml (adrenals). Monoamines were extracted as described by Sharman (1971). Throughout the extraction, the samples were kept at 4°C and were mixed with a Rotamixer following each addition of reagent. Two aliquots (5 ml) of each homogenate were pipetted into nylon centrifuge tubes and to one aliquot amines were added (2 µg noradrenaline/5 ml adrenal homogenate or 1 µg noradrenaline and 1 µg 5-HT/5 ml brain homogenate) to estimate their recovery by the extraction procedure. Proteins were removed by the addition of perchloric acid (71-73% w/w) (30 µl/ml) and centrifugation at 15,000 g for 5 minutes at 0°C. The clear supernatants were decanted into glass centrifuge tubes and the pH adjusted to 4 with a dilute solution of

potassium carbonate. The extracts were left for 10 minutes to allow CO_2 to escape before centrifugation at 900 g for 5 minutes to remove potassium perchlorate.

Purification of the tissue extracts:-

A cation exchange resin (Amberlite CG50, ammonium form) was used for the purification of the extracts. The resin was prepared as described by Bartlet & Gilbert (1971). The resin (hydrogen form) was stirred 3 times with 1 M HCl (3 vols) for 30 minutes and rinsed with distilled water until the washing was free from chloride. To convert to the ammonium form, the resin was stirred 3 times with 3 M NH_4OH (3 vols) for 30 minutes. The ammonium form of the resin was washed first with distilled water until the pH of the effluent fell to about 9 and then with 0.2 M ammonium acetate adjusted to pH 7.5 with 2 M NH_4OH, and was stored at $5^{o}C$ under the ammonium acetate buffer.

The supernatants were loaded on the Amberlite columns (3 × 1 cm), which were placed in the dark and the solutions allowed to flow through by gravity at a rate not exceeding 0.25 ml/min. They were washed with 0.02 M ammonium acetate (15 ml) adjusted to pH 7.5 with 0.5 M ammonium hydroxide, and then with 0.05M sulphuric acid (3 ml) before eluting the amines with 0.5M sulphuric acid (5 ml). The brain eluates were divided into two portions for the estimation of 5-HT and catecholamines, respectively. The pH of the eluates used for the estimation of catecholamines

were adjusted to 4 with solid sodium bicarbonate, and stored overnight at -20°C.

Aliquots (5 ml) of solutions of 5-HT (0.05, 0.10 and 0.50 µg/ml) and noradrenaline (0.02, 0.1 and 0.25 µg/ml) in 0.04 M HCl containing ascorbic acid (2 mg/ml) were carried through the whole extraction procedure with each batch of brain homogenates. These served as internal standards in the estimation of the amines. Internal standards of noradrenaline (0.5, 2,0 and 4.0 µg/ml) were prepared with each analysis of adrenal homogenates.

Estimation of monoamines:-

An Aminco-Bowman spectrofluorimeter was used to measure the fluorophors, and the wavelengths quoted are uncorrected values. The estimations were carried out in test tubes washed with detergent and chromic acid and thoroughly rinsed with distilled water. The contents of the tubes were mixed with a Rotamixer after each addition of reagent.

5-HT:-

Concentrated HCl (1 ml) containing ascorbic acid (0.5 mg/ml) was added to the eluate (2 ml) to make it 3M with respect to HCl, and 5-HT estimated by measuring the peak in the activation scan at 290 nm, with fluorescence recorded at 535. With each batch of analyses an "extraction blank" was prepared by substituting water for brain homogenates in the extraction, and the blank

values subtracted from the readings before calculating the amounts of 5-HT.

Adrenaline and noradrenaline:-

Adrenaline and noradrenaline were estimated differentially by the THI method (von Euler and Floding, 1955), utilizing differences in the oxidation rates of the amines at a low pH.

One aliquot of eluate (1 ml) was mixed with 1M sodium acetate buffer adjusted to pH 6.5 (0.5 ml), and diluted to 3.0 ml with distilled water. The tube was placed in the dark for the oxidation of adrenaline and noradrenaline with potassium ferricyanide (0.25% w/v) (0.1 ml). After 3 minutes, ascorbic acid (0.2% w/v) (0.1 ml) and sodium hydroxide (20% w/v) (0.9 ml) containing 1,2-diaminoethane (2% w/v) were added, and the mixture kept in the dark 7 minutes for fluorophor formation. After dilution to 5.0 ml with distilled water, the fluorophors formed from adrenaline and noradrenaline were estimated by measuring the peak in the fluorescence scan between 510 and 520 nm, with activation at 410 nm.

A second aliquot of eluate (1 ml) was mixed with 1M sodium acetate buffer adjusted to pH 3.5 (0.5 ml), zinc sulphate (0.5% w/v) (0.1 ml) and distilled water to 3.0 ml. At pH 3.5 only adrenaline was rapidly oxidized with potassium ferricyanide, and treatment with ascorbic acid and sodium hydroxide containing 1,2-diaminoethane produced a fluorophor from adrenaline and only 4.9 ± 0.4% of the

noradrenaline (mean ± s.e.m. n = 6).

With each batch of analyses an "extraction blank" was prepared by substituting water for brain homogenates in the extraction. The blank values were subtracted from the readings before calculating the amounts of adrenaline and noradrenaline present by means of the following equations:-

$$X = \frac{100(A-B)}{(100-n)}$$

$$Y = A - \frac{100(A-B)}{(100-n)}$$

where X and Y = flourescence due to noradrenaline and adrenaline in the eluate, respectively.
A and B = fluroescence readings of the eluates at pH 6.5 and 3.5, respectively.
n = % noradrenaline oxidized at pH 3.5.
m = ratio of fluorescence intensities of adrenaline/noradrenaline at pH 6.5.
[The values for n and m were 4.9 ± 0.4 (6) (mean ± s.e. mean, number of observations) and 1.7 ± 0.0 (6), respectively.]

A graph relating fluorescence to noradrenaline (μg) was constructed from the internal standards. The noradrenaline equivalents of X and Y were read from this graph, and the value so obtained from Y divided by m to obtain the equivalent in terms of adrenaline.

Validation of the method for the estimation of adrenaline and noradrenaline in a mixture

The following experiments were carried out to test the validity of the differential estimation of adrenaline and noradrenaline when present in a mixture.

1) Determination of the rates of oxidation of adrenaline and noradrenaline in the presence of $ZnSO_4$ at pH 3.5.
2) The effect of pH on the rate of oxidation of adrenaline and noradrenaline.
3) Relative fluorescence intensities of adrenaline and noradrenaline at pH 6.5.
4) Relative fluorescence intensity of dopamine in the THI reaction.
5) Recovery of amines from the CG50 columns and from the total extraction procedure.

1) Determination of the rates of oxidation of adrenaline and noradrenaline in the presence of $ZnSO_4$ at pH 3.5

It has been reported that $ZnSO_4$ increases the rate of oxidation of adrenaline at pH 3.5, the effect being dependent on the amount of the catalyst used (von Euler and Floding, 1955). Preliminary experiments were conducted to find out the optimum concentration of $ZnSO_4$ which would give the maximum rate of oxidation of adrenaline (1 µg/ml) at pH 3.5. $ZnSO_4$ was added to the oxidation mixtures at concentrations ranging from 0.007 - 0.833 mg/ml and the relative fluorescence intensities of adrenaline (1 µg/ml)

measured after 3 minutes oxidation with potassium ferricyanide. The results of this experiment (fig. 4) showed that about maximal fluorescence intensity was obtained using $ZnSO_4$ at a final concentration of 0.17 mg/ml, which was not significantly different from the fluorescence intensity obtained with 0.83 mg/ml ($p > 0.1$).

To determine whether full oxidation of adrenaline occurred in 3 minutes at pH 3.5 in the presence of $ZnSO_4$ (final concentration 0.17 mg/ml), experiments were carried out in which oxidation was stopped with ascorbic acid 3', 4', 5', 6', 7', 8', 9' and 10' after adding potassium ferricyanide. The results of this experiment (fig. 5) showed that adrenaline (1 µg/ml) was completely oxidized in 3 minutes giving maximal fluorescence. Noradrenaline (1 µg/ml), under the same conditions gave a relatively low fluorescence intensity which increased slowly with time.

2) <u>The effect of pH on the oxidation rate of adrenaline and noradrenaline</u>

To determine the amount of adrenaline and noradrenaline present in a mixture, it was necessary to obtain a reliable value for the percentage of noradrenaline which is oxidized in 3 minutes at pH 3.5 in the presence of $ZnSO_4$. The two amines were reacted by the THI method at pH 6.5 and at pH 3.5 in the presence of the catalyst with 3 minutes of oxidation, and the fluorescence intensity recorded. It was found that the percentage of noradrenaline oxidized at pH 3.5 (with 3 minutes oxidation) was 4.9 ± 0.4 (6).

No significant difference was found between the fluorescence intensities of adrenaline at pH 3.5 and pH 6.5 ($p > 0.1$), indicating that this amine was fully oxidized under both sets of conditions.

3) The relative fluorescence intensity of adrenaline and noradrenaline at pH 6.5

For the estimation of adrenaline and noradrenaline present in a mixture, the relative fluorescence of equal amounts of the two amines (m) at pH 6.5 must be known. To obtain a reliable value for (m), a number of experiments were performed using concentrations of the two amines varying from 0.05 µg base/ml to a 5 µg base/ml. The regression of fluorescence upon amine concentration was calculated by the method of least square and (m) was found to have a value of 1.7 (fig. 6).

4) Relative fluorescence intensity produced by dopamine in the THI reaction

Dopamine is present in the avian brain (Holzbauer & Sharman, 1971), and in small amounts in the adrenal gland of some species like ox and sheep (Shepherd & West, 1953). To find out whether dopamine would interfere with the estimation of adrenaline and noradrenaline, an experiment was performed in which relatively high concentration of dopamine (25, 50 and 100 µg/ml) were reacted by the THI method at pH 6.5 and pH 3.5, with oxidation time of 3 minutes. For comparison, three concentrations of noradren-

aline and adrenaline (0.25, 0.5 and 1.0 µg/ml) were simultaneously reacted at pH 6.5 and 3.5, respectively. Some of the results of this experiment (fig. 7) show that dopamine (100 µg/ml) produced little or no fluorescence in the THI reaction, thus, in physiological amounts it is unlikely to interfere with the estimation of catecholamines.

5) Recovery of monoamines from the CG50 columns and from total extraction procedure

The recovery of noradrenaline (1 µg/ml) and 5-HT (1 µg/ml) from the CG50 columns and after carrying them through the extraction procedure was investigated.

Aliquots (5 ml) of 5-HT or noradrenaline (dissolved in distilled water) were put directly onto the resin columns, which were washed with 0.02 M ammonium acetate buffer (15 ml) and 0.05 M H_2SO_4 (3 ml) before eluting the amines with 0.5 M H_2SO_4 (5 ml).

Other aliquots (5 ml) of 5-HT or noradrenaline in 0.04 M HCl containing ascorbic acid (2 mg/ml) were mixed with perchloric acid, 72% w/v (0.15 ml) and the pH adjusted to 4 with K_2CO_3. After allowing the solutions to stand for 10 minutes to enable CO_2 to escape, they were centrifuged at 900 g for 5 minutes at 5°C. The supernatants were decanted into the resin columns and eluted as described above.

The results of this experiment (table 2) showed that, after direct addition to the columns, 5-HT and noradrenaline

were eluted with a percentage recovery of 93.1 ± 2.1 (mean ± s.e.m. n = 5) and 81.8 ± 1.4%, respectively. These percentage recoveries were significantly less than 100% ($p < 0.01$ for 5-HT and < 0.001 for noradrenaline). When the amine solutions were carried through the extraction procedure, the recoveries of 5-HT and noradrenaline were 89.8 ± 0.7% and 78.4 ± 1.8%, respectively. These recoveries were not statistically different from those obtained after direct addition of 5-HT and noradrenaline to the columns ($p > 0.1$ for both amines).

In 24 experiments, the recovery of 5-HT (1 µg) and noradrenaline (1 µg) added to brain homogenates was 81.2 ± 2% (mean ± s.e.m.) and 104.0 ± 5.0% respectively. It was noted that the recovery of noradrenaline from the tissue homogenates was significantly greater than from the columns ($p < 0.001$) or total extraction procedure ($p < 0.001$), which suggests the presence of a substance(s) in tissue protecting noradrenaline against losses.

6) Characteristics of the spectra of tissue extracts

The emission spectra obtained from the adrenal extracts at pH 6.5 and 3.5 had peaks at 510 and 520 nm, respectively, whichwere the emission peaks of the authentic noradrenaline (> 1 µg/ml) and adrenaline. In a number of experiments attempts were made to estimate the catecholamines in the hearts and brains of chickens. The peaks of the emission spectra in these tissue extracts (490 nm) was similar to that of internal standards of noradrenaline

at relatively low concentrations (0.05 and 0.1 µg/ml) (fig. 8A). This observation was also noted with the heart and brain extracts in rat and G. pig. It seems that the wavelength of the peak in the emission spectra of noradrenaline shifted from 485 to 492 nm as the amount of the catecholamine increased from 0.05 to 0.2 µg/ml (fig. 8B).

In some preliminary experiments the Dowex-50 resin was used for the purification of catecholamines. The amines were recovered from this resin to the same extent as the amberlite CG50. As the latter may be used for the purification of both catecholamines and 5-HT, its use in all subsequent experiments was preferred.

Measurement of the vasopressor and vasodepressor actions of amines in anaesthetized chickens

Pullets aged 17-19 weeks were anaesthetized with phenobarbitone sodium (180 mg/Kg) which was injected into a wing vein as a 10% w/v solution in 0.9% w/v aqueous NaCl. Body temperature was maintained by means of a heated operating table and an overhead warming lamp. An internal carotid artery was cannulated with nylon tubing (Portex 800/200/100) filled with heparin (7,000 units/ml), and connected to a Devices pressure transducer for measuring the arterial blood pressure on a Devices pen recorder. A wing vein was cannulated with a medical vinyl tubing (v-3) (Bolab Inc., U.S.A.) for the administration of drugs. In each preparation, noradrenaline acid tartarate was given intravenously at doses increasing from 0.015 to 4.0 µg/Kg in geometric progression. Tyramine HCl (20-1280 µg/Kg) was given following the same protocol. In other preparations 5-hydroxytryptamine creatinine sulphate (0.25-2.0 µg/Kg), histamine dihydrochloride (1.6-12.8 µg/Kg) and tryptamine HCl (5-40 µg/Kg) were injected intravenously and the depressor responses recorded. All the doses, in a volume not exceeding 1 ml, were injected at 5 minute intervals and washed into the circulation with 0.9% w/v aqueous NaCl (1 ml).

In some further experiments tyramine was given intraduodenally in anaesthetized preparations of cockerels (9-10 weeks). A longitudinal incision was

made in the right abdomen 2.5 cm from the midline and the ascending or descending duodenum opened for the insersion of a catheter with its tip pointing distally. Tyramine HCl was injected intraduodenally at a dose of 5 mg/Kg and further doses were given when the blood pressure had returned to the control level.

Measurement of feed intake and growth of birds

Before starting a feed trial, the birds were distributed at random into experimental groups, provided ad libitum with food and water and left for 4-7 days for acclimitization to the "new" surroundings. The birds and their feed intake were weighed daily during the acclimitization period to accustom them to handling and to ensure that a normal pattern of feeding and growth had been attained. A top pan balance (AVERY), measuring to the nearest gramme, was used.

To achieve a reasonable degree of accuracy in measuring the feed intake of birds, food was given in different containers according to the size of the birds, viz. in experiments where chicks, turkey poults or ducklings were used, food was given in small bowls (12 cm. in diameter) covered with wire mesh to prevent spillage associated with pecking activity and direct contact with the birds. When chickens (8 weeks or older) were used, the food was given in troughs with edges turned inwards to minimize spillage. The container was weighed daily, before and after replenishment with food.

Measurement of blood lactate and pyruvate

The enzymatic method of Marbach & Weil (1967) was used to measure the concentrations of lactate and pyruvate in blood.

Principle of the method:-

In the presence of nicotine adenine dinucelotide (NAD^+), lactic dehydrogenase (LD) will oxidize lactate to pyruvate. The reduced form of nicotine adenine dinucelotide (NADH) produced in the reaction may be measured spectrophotometrically at 340 nm, and this serves as a measure of the lactate concentration.

$$\text{Lactate} + NAD^+ \underset{\text{LD, pH 9.0 to 9.6}}{\rightleftharpoons} \text{pyruvate} + NADH + H^+$$

At pH 9.0 to 9.6 and in the presence of NAD^+ and hydrazine (to trap pyruvate), the equilibrium of the above reaction favours the oxidation of lactate.

The reaction involved in the determination of pyruvate is essentially the reverse of the reaction used in the lactate procedure.

$$\text{pyruvate} + NADH + H^+ \underset{\text{LD, pH 7.5}}{\rightleftharpoons} \text{lactate} + NAD^+$$

At pH 7.5, the equilibrium constant of the above reaction favours the formation of lactate.

Blood collection and deproteinization:-

About 1 ml of blood was collected from a wing vein by means of a heparinized syringe fitted with a 21 G needle, and delivered as quickly as possible into a

chilled tared centrifuge tube containing 5% w/v metaphosphoric acid (3 ml). The tubes were stoppered and the contents mixed quickly by inverting 4 times. The samples were allowed to stand for 5 minutes at room temperature before being reweighed and centrifuged at 900 g for 30 minutes at 5°C.

Lactate determination:-

An aliquot of supernatant (0.1 ml) was mixed with glycine-hydrazine buffer, pH 9.0 (2 ml), 0.3% w/v LDH in 0.85% w/v NaCl (0.03 ml) and 0.027 M NAD (0.2 ml) in a 1 cm cuvette, and left for 14 minutes at room temperature before measuring the absorbence of the mixture at 340 nm. A standard and a blank were also prepared in which the deproteinized blood was replaced with 1.0mM lactate in 3% w/v metaphosphoric acid (0.1 ml) or 3% w/v metaphosphoric acid (0.1 ml), respectively. The concentration of lactate in the sample was obtained from the expression:

$$\left[\frac{\text{A test - A blank}}{\text{A standard-A blank}} \times \text{standard concentration (mM)} \times D\right]$$

A, absorbance at 340 nm.

D, the dilution of the blood sample in the metaphosphoric acid and is equal to $\frac{\text{weight of blood + (3 × 1.06)}}{\text{weight of blood}}$

pyruvate determination:-

A portion of supernatant (1.0 ml) was mixed with 0.75 M Tris buffer, pH 7.4 (0.5 ml) and 0.013 M NADH in 1% w/v sodium bicarbonate (0.03 ml) in a cuvette (1 cm). The

absorbance of the mixture was measured at 340 nm, after setting the spectrophotometer scale to zero with a 1 cm cuvette containing 0.0002 M potassium dichromate solution in the light path. Then 0.3% w/v LDH (0.03 ml) was added and the absorbance measured again after exactly 2 minutes. A standard and a blank were also prepared in which the deproteinized blood was replaced with 0.05 mM sodium pyruvate in 3% w/v metaphosphoric acid (1 ml) or 3% w/v metaphosphoric acid (1.0 ml), respectively. The concentration of pyruvate in blood was calculated from the expression:-

$$\left[\frac{\Delta A \text{ test} - \Delta A \text{ blank}}{\Delta A \text{ standard} - \Delta A \text{ blank}} \times \text{standard concentration (mM)} \times D\right]$$

Measurement of erythrocyte transketolase activity

Transketolase (TK) is an enzyme found in nearly all tissues of the body. It has a requirement for Mg^{++} and thiamin pyrophosphate (TPP), and the measurement of its activity in lysed erythrocytes is considered a useful test of the thiamin status of animals.

TK activity was measured in chicken erythrocytes by a method described by Brin (1974).

Basis of the method

TK, in conjunction with its required coenzyme TPP catalyzes two reactions in the pentose phosphate cycle (PPC).

ribose-5-phosphate + xylulose-5-phosphate $\xrightleftharpoons{\text{TK-TPP}}$ sedoheptulose-7-phosphate+glyceraldehyde-3-phosphate.

erythrose-5-phosphate + xylulose-5-phosphate $\xrightleftharpoons{\text{TK-TPP}}$ fructose-6-phosphate+glyceraldehyde-3-phosphate.

In this method the activity of part of the PPC is estimated by measuring the rate of conversion of ribose-5-phosphate into hexose phosphates. If it is assumed that the TK reactions are rate limiting, then this activity gives a measure of the activity of TK. The demonstration of the dependence of the reaction rate on TPP concentration (figure 25) is an indication that transketolase is the limiting enzyme in the assay (Brin 1974). The TK reaction is stopped by deproteinization after a fixed time and a reaction product determined spectrophotometrically by the authrone method.

Collection and lysing of erythrocytes

Blood was withdrawn from a wing vein by means of a heparinized syringe fitted with a 21G needle and put into a tared centrifuge tube. The blood was spun down in a refrigerated centrifuge (5°C) at 900 g for 20 minutes and the plasma discarded. After drying the outside of the tubes, the cells were weighed and mixed with an equal volume of distilled water. The specimens were stored overnight in the frozen state (-20°C) to affect complete haemolysis.

Incubation procedure

An aliquot of haemolysate (0.5 ml) was pipetted into 3 tubes for the 'test', 'tissue blank' and 'TPP effect' measurements. To the 'test' and 'tissue blank' a saline-phosphate buffer (0.45 ml and 0.65 ml, respectively) was added, and 0.26 mM TPP in the same buffer (0.45 ml) was added to the tube for the 'TPP effect'. The contents of the tubes were mixed and incubated with shaking in a water bath at 38°C for 30 minutes. Then the substrate, 0.47 mM ribose-5-phosphate (0.2 ml) was added with mixing to the 'test' and 'TPP effect' reaction mixtures. All three tubes were incubated for 60 minutes at 38°C with shaking before stopping the reaction with 7.5% w/v trichloracetic acid (6 ml) and centrifuging off the precipitate at 900 g for 20 minutes at 5°C.

The 'anthrone procedure' was employed for the spectrophotometric estimation of the 'reaction product'.

Aliquots of the supernatant (1.0 ml) were mixed with 10 ml of anthrone reagent (2.5 mM anthrone and 0.13 M thiourea in 66% w/v H_2SO_4), heated in a boiling water bath for 10 minutes, cooled for 5 minutes in a cold water bath and allowed to stand in the dark for 20 minutes. Aliquots (1.0 ml) of standard glucose solution (0.55 mM in 0.75% w/v trichloroacetic acid) and reagent blank (0.75% w/v trichloroacetic acid) were carried through the 'anthrone procedure' described above.

The absorbance (A) of the solutions was read in a spectrophotometer at a wavelength of 620 nm, with the reagent blank set at 0. The activity of transketolase was obtained from the expression:-

$$\left[\frac{(A_{sample} - A_{tissue\ blank}) \times \left\{ \begin{matrix} \text{concentration} \\ \text{of standard} \\ \text{solution} \\ (\mu\text{mol/ml}) \end{matrix} \right\} \times 7.15 \times 1000}{A_{standard} \times 60 \times 1.0 \times 0.5} \right] \text{ units/litre}$$

The 'TPP effect' which refers to the increase in transketolase activity in a deficient haemolysate due to the addition of TPP before incubation, expressed as a percentage of that in the deficient haemolysate without the addition of TPP, was obtained from the expression:-

$$\text{TPP effect}(\%) = \frac{(\text{Test with TPP}) - (\text{Test without TPP})}{(\text{Test without TPP})} \times 100$$

Estimation of the Oxygen Consumption in Chicken Tissue Homogenates

The oxygen consumption of tissue homogenates was estimated manometrically using the Gilson Respirometer, which is a direct-reading constant pressure instrument that measures the oxygen consumed by the incubates.

Preparation of tissues

Chickens (aged 9 weeks) were decapitated and the tissues rapidly removed and homogenized in 0.067 M phosphate buffer, pH 7.4.

Incubation procedure

Each incubation mixture consisted of homogenate (2 ml) (equivalent to 100, 200 or 400 mg tissue) and 0.067 M phosphate buffer, pH 7.4 (2 ml), and was placed in the main compartment of a reaction flask. A strip of filter paper soaked in IN KOH (0.3 ml) was placed in the centre well of the flask to absorb CO_2. Flasks were assembled on the apparatus without stoppers on the side arm and oxygen blown through for 5 minutes to displace all air in the system. The flasks were then stoppered and immersed in water at 37°C with shaking. When equilibrium was reached (i.e., the level of the fluid in the u-tubes was absolutely steady when the by-pass valves between the arms of the u-tubes were turned off), a note of time was made and the micrometer pistons adjusted so that

the pressure remained constant in the flasks, readings being taken at 3, 6, 9, 12, 15, 20, 25 and 30 minutes.

The oxygen consumption of the tissues at the 20th min was corrected to values under standard temperature and pressure (S.T.P.) using tables (Scientific Tables, Geigy) and expressed as $\mu l O_2$/g tissue/min from the expression :

$$\left(\frac{\text{Volume of oxygen (S.T.P.)} \times 1000}{\text{Weight of tissue (mg)} \times 20} \right)$$

Estimation of cholesterol concentration in plasma and adrenals of chickens

The method of Zlatkis, Zak & Boyle (1953) was used for the estimation of cholesterol in chicken plasma. A slight modification of this method was adopted for the estimation of cholesterol concentration in the adrenals as described by Knobil, Hagney, Wilder & Briggs (1954).

1. Estimation of total plasma cholesterol

Blood was collected from a wing vein in a heparinized syringe and put into a centrifuge tube. The blood was spun down at 900 g for 20 minutes at 5°C. An aliquot of plasma (0.1 ml) was pipetted into a test tube containing glacial acetic acid (3 ml). A 'colour reagent' (0.1% w/v ferric chloride in concentrated H_2SO_4) (2 ml) was then added with mixing in a rotamixer for 30 seconds. A light brown colour first appeared which changed to an intense purple within a minute. The absorbance of the solution was measured spectrophotometrically at a wavelength of 560 nm. Aliquots (0.1, 0.2, 0.3, 0.4 and 0.5 ml) of a standard cholesterol solution (1 mg/ml in glacial acetic acid) were made up to 3.1 ml with glacial acetic acid and water (0.1 ml) and reacted with the 'colour reagent', and these served as standards in the estimation of plasma cholesterol concentration. A reagent blank was also prepared with each batch of analyses, which contained glacial acetic acid (3 ml), distilled water (0.1 ml) and the 'colour reagent' (2 ml).

The concentration of cholesterol in plasma was calculated from the calibration curve (fig. 9) constructed from the cholesterol standard solutions in each experiment.

2. Estimation of total adrenal cholesterol

Chickens were decapitated and the adrenals rapidly dissected out, weighed and homogenized in glacial acetic acid (10 ml). The homogenates were filtered through Whatman filter paper (No. 1), and an aliquot of the filtrate (0.5 ml) mixed with glacial acetic acid (3 ml). The 'colour reagent' (2.5 ml) was then added with mixing for 30 seconds. The intensity of the resulting purple colour was measured in a spectrophotometer at 560 nm.

With each batch of analyses, a fresh calibration curve was constructed, which related the absorbences at 560 nm to amount of cholesterol.

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Estimation of glucose concentration in chicken plasma

Glucose concentration in chicken plasma was estimated by a modification of the glucose oxidase method (Huggett & Nixon, 1957; Trinder, 1969).

Basis of the method

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconate} + H_2O_2$$

$$H_2O_2 + \text{aminophenazone} \xrightarrow{\text{peroxidase}} \text{coloured complex} + H_2O$$

Procedure

Blood was withdrawn from a wing vein of chickens (8 weeks old) using a heparinized syringe fitted with a 21 G needle, and spun down at 900 g for 20 minutes at 5°C to separate plasma. An aliquot of plasma (0.1 ml) was deproteinized with 0.1 M sodium tungstate (2 ml) and centrifuged at 900 g for 20 minutes at 5°C. A portion of the supernatant (0.1 ml) was mixed with a 'reagent' (2 ml) and incubated at 37°C for 30 minutes with shaking. [The 'reagent' contained peroxidase (80 units/mg) (3.3 mg), glucose oxidase (129,200 units/g) (8.5 mg), phenol (100 mg) and 4-aminophenazone (30 mg), in 100 ml of 0.01 M sodium phosphate buffer, pH 7.0].

In each experiment, aliquots (0.1 ml) of glucose standards (100, 200 and 500 µg/ml) in 0.1 M sodium tungstate, and blank (0.1 sodium tungestate), were also mixed with the 'reagent' (2 ml) and incubated at 37°C for 30 minutes with shaking.

At the end of the incubation period, the red purple colour was read in a spectrophotometer at a wavelength of 510 nm. The absorbance of plasma supernatants were converted to concentrations of glucose from a calibration curve of glucose solutions (fig. 10).

Estimation of ascorbic acid concentration in the adrenal gland

Ascorbic acid was estimated in the adrenal glands of chickens by the method of Maickel (1960), which depends on reduction of Fe^{+++} by ascorbic acid and estimation of Fe^{++} as the red-orange, α - α'-dipyridyl complex.

The chickens were decapitated and the adrenals rapidly removed, weighed and homogenized in 5% w/v trichloroacetic acid (5 ml) with 6 strokes in a glass homogenizer. The homogenates were centrifuged at 900 g for 5 minutes at $5^{o}C$. An aliquot of the supernatant (1 ml) was transferred to a test tube and mixed with 85% H_3PO_4 (0.3 ml), followed by 0.5% w/v aqueous α-α'-dipyridyl (5.0 ml), and 1% w/v aqueous $FeCl_3$ (1.0 ml). The tubes were then allowed to stand at room temperature for 10 minutes before reading the absorbance of the red-orange Fe^{++}-α-α'-dipyridyl complex in a spectrophotometer at 525 nm using water as a zero reference.

A standard solution of ascorbic acid (25 µg/ml in 5% w/v trichloroacetic acid) was prepared with each estimation of adrenal ascorbic acid and carried through the procedure described above and its absorbence was used in calculating the amount of ascorbic acid in the adrenal supernatant.

Measurement of arterial blood pressure in conscious chickens

Pullets aged 17-19 weeks were divided at random into two groups; one group received an unmedicated (control) diet, and the other, furazolidone in the feed at a concentration of 0.04% w/w for 10 days.

Anaesthesia

Inhalation anaesthesia was induced with halothane 5% v/v in oxygen for one minute, and maintained during the operation by halothane 2% v/v. The anaesthetic was delivered from a vaporizer (Fluote 3) at a rate of 500 ml/min through a narrow polyethylene tube inserted in the trachea.

Operation

After plucking the feathers from the skull and along the medial line in the crop region, the anaesthetized bird was secured on its back and kept warm by an overhead lamp and a heating table. A longitudinal incision was made along the medial line and the muscles were separated to expose the internal carotid arteries. A carotid artery was cannulated with a nylon catheter (Portex, 800/200/100) filled with heparin (7,000 i.u./ml) for measuring blood pressure. Using a metal probe the catheter was drawn up the neck under the skin and exteriorized near the base of the skull. A sawn-off syringe needle (25G) was inserted at the end of the exteriorized catheter and a catheter adaptor fitted into the needle hub.

Post-operative care

The chickens were conscious within 10 minutes of stopping the anaesthesia and were kept in draught-free ventillated 'recovery' boxes for at least 3 hours before measuring the arterial blood pressure.

Measurement of arterial blood pressure in conscious chickens

Each bird was placed in a ventillated wooden box with the minimum of excitement and was left undisturbed for 30 minutes before recording blood pressure. The exteriorized cannula was connected to a blood pressure transducer which was attached to a pressure amplifier and a pen recorder (Devices 3552). The mean arterial blood pressure was obtained from 3-4 blood pressure recordings made at 30 minute intervals.

Determination of packed cell volume and haemoglobin content of chicken blood

Packed cell volume (PCV)

PCV was measured in chicken blood by the micro-haematocrit method as described by Dacie & Lewis (1975). Blood was collected from a wing vein in a heparinized syringe, and mixed in a centrifuge tube. The blood was introduced to the microhaematocrit capillary tubes (75 mm x 1.0 mm) by capillarity, leaving at least 15 mm unfilled. The tip of the capillary was sealed with plasticine and the tubes placed in a microhaematocrit centrifuge (Hawksley, England) and spun down at 12,000 g for 5 minutes. After centrifugation, the capillary tube was placed in a reading device (Hawksely Reader, Cat. No. A.802), and PCV read directly from the scale.

Haemoglobin (Hb) content

The cyanomethaemoglobin (HiCN) method described by Dacie & Lewis (1975) was employed for the estimation of Hb in chicken blood. In this method blood is diluted with a solution containing potassium ferricyanide and potassium cyanide to convert Hb to HiCN. The absorbance of HiCN is then measured in a spectrophotometer.

The cyanide reagent used in the present experiments consisted of potassium ferricyanide (200 mg) and

potassium cyanide (50 mg) dissolved in 1 litre of 0.1 M phosphate buffer solution, pH 7.2, the buffer being intended to overcome the formation of a red precipitate from the fowl's blood (Beuving & Teunis (1974)).

Chicken blood (20 µl) was added to the cyanide reagent (5 ml) and mixed with a rotamixer. The colourless precipitate formed was centrifuged down (900 g for 5 minutes at 5°C) immediately after addition of the blood to the cyanide reagent. The supernatant was left for 10 minutes at room temperature and its absorbance was read in a spectrophotometer at a wavelength of 540nm, against a blank of cyanide reagent. A standard solution of HiCN was also read against the blank. Haemoglobin content of the blood (g/100 ml) was obtained from the expression:-

$$\left[\frac{\text{absorbence of test sample}}{\text{absorbence of standard}} \times \frac{\text{concentration of standard (mg/100 ml)} \times \text{dilution factor}}{1000}\right]$$

Determination of the Activity of Aspartate Transaminase in Chicken Plasma

The activity of aspartate transaminase (AST, EC 2.6.1.1) was determined in chicken plasma by the method described by Wilkinson, Baron, Moss & Walker (1972), which measures the rate at which absorbance at 340 nm decreases as NADH is oxidized to NAD.

Principle of the method

The reactions involved in the determination of AST activity are as follows :

$$\text{aspartate} + \text{2-oxoglutarate} \xrightleftharpoons{\text{AST}} \text{glutamate} + \text{oxaloacetate} \quad (1)$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons[\text{dehydrogenase}]{\text{malate}} \text{malate} + \text{NAD}^+ \quad (2)$$

The oxaloacetate produced by the aspartate transaminase serves as substrate for malate dehydrogenase by which it is reduced to malate in the presence of NADH, which is simultaneously oxidized. NADH has an absorbance peak at 340 nm which is not shown by the oxidized form, and the decrease in absorbence at this wavelength provides a means for the measurement of the aspartate transaminase activity.

Procedure

Blood was withdrawn from a wing vein by means of a heparinized syringe fitted with a 21 G needle and spun down at 900 g in a refrigerated centrifuge (5°C) for 20 min to

separate plasma. An aliquot of plasma (0.2 ml) was added with mixing to 0.1 M phosphate buffer, pH 7.2 (1.3 ml), 0.375 M L-aspartate (1.0 ml), 3.53 mM NADH (0.2 ml) and malate dehydrogenase (5 u/ml) (0.1 ml), and the mixture incubated for 15 min at 25°C. The mixture was transferred to a cuvette (1 cm) and the reaction started by the addition of 0.1 M 2-oxoglutarate (0.2 ml) followed by mixing. The decrease in absorbance was then measured at 340 nm every minute for a period of ten minutes. For the purpose of calculation the readings obtained at the first three minutes were excluded (as the reaction does not obey zero order kinetics during this period).

The rate of change in absorbance/minute was measured ($\Delta A/min$) and the aspartate transaminase activity was calculated in u/l from the expression :

$$\text{enzyme activity} = \Delta A/min \times 2400 \text{ u/l}$$

Statistical analysis of results

When three or more observations were made, the values reported in the text, tables and figures are the means with standard errors. The significance of the difference between the means was estimated by the t-test and probability (P) values are given. A value of $P < 0.05$ is regarded as significant. Regression lines were calculated by the method of least squares.

Chapter I

The effect of furazolidone on MAO activity

Results

The effect of age on MAO activity in the chicken

Many workers have shown that the activity of MAO in tissues of avian species increases with age (Ignarro & Schiedeman, 1968; Watts, Mendes, Reilly & Krop, 1969; Fowler & Callingham, 1977). At hatching, the activity of MAO in the brains of chicks, ducklings and pigeons has been shown by Watts et al (1969) to be about 60%, 90% and 34%, of the adult level, respectively. An experiment was conducted in which MAO activity was measured in organs of day-old and 8-weeks old chickens. The results of this experiment (table 3)showed that the activity of the enzyme in the duodenal mucosa, liver and heart of day-old chicks was significantly lower than in 8 weeks old chickens ($P < 0.02 - < 0.001$). No significant difference was found in the activity of MAO in the brains of chickens at these ages ($P > 0.2$).

In some of the present experiments, it was necessary to use birds at one particular age in order to avoid age variation in MAO activity.

The effect of furazolidone feeding on MAO activity in chickens, ducklings and turkey poults

When furazolidone was given in the food at a concentration of 0.04% w/w for 10 days, the activity of MAO was reduced significantly in the duodenal mucosa, heart and brains of chickens, ducklings and turkey poults. Only in

the duckling was a significant inhibition of MAO activity found in the liver ($P < 0.01$) (table 4). These experiments were repeated twice with chickens and once with ducklings and similar results were obtained.

In another experiment, the recovery of the enzyme activity after discontinuing the medicated feed was investigated in the chicken. This experiment confirmed the effect of feeding furazolidone on MAO activity, which was inhibited in the duodenal mucosa ($P < 0.001$), heart ($P < 0.02$) and brain (0.02) and not in liver ($P > 0.1$) (fig.11). Seven days after discontinuing furazolidone in the feed a significant recovery of MAO activity was only evident in the duodenal mucosa ($P < 0.002$). Fourteen days after discontinuing the drug, the recovery of MAO activity was complete in each organ, there being no significant difference from the controls ($P > 0.1$ for each organ).

The distribution of MAO activity in the mucosa of the alimentary tract of chickens aged 10 weeks is shown in fig. 12. A very high activity was found in all parts of the alimentary mucosa, except in the oesophagus, and even here the activity was of the same order as that found in the liver and brain of birds aged 8 weeks. Although MAO activity in chicken organs increases with age, a comparison between birds aged 8 and 10 weeks seems valid as the activity in the duodenal mucosa did not differ significantly at these ages ($P > 0.1$).

The inhibition of MAO activity in the alimentary mucosa of the chicken (except that in the rectum) following the treatment with furazolidone at the therapeutic dose (0.04% w/w in the feed for 10 days) was more than that in the heart, brain or liver (fig. 13). The enzyme inhibition was most marked in the caecal mucosa ($P < 0.02$ - <0.001 compared with other organs), and least in the liver where none was found ($P > 0.1$).

The effect of furazolidone by crop tube or intramuscular injection on MAO activity in chickens.

Furazolidone was administered to chickens at a dose of 200 mg/kg body weight, which is about half the reported LD_{50} in this species (Radchuck, 1965). The drug administered by crop tube inhibited MAO activity in the chicken liver as well as in duodenal mucosa, heart and brain (table 5). The time course of inhibition was different in various organs, however, being maximal in the liver 12 hours after drug administration and in the duodenal mucosa and brain after 24 hours. In contrast, furazolidone injected intramuscularly did not affect MAO activity in any of the organs studied ($P > 0.1$ for each organ, 6 birds) (fig. 14).

Potency of furazolidone as an MAO inhibitor in chicks, ducklings and turkey poults

MAO activity was measured in chicks, ducklings and turkey poults aged 2 - 12 days, 24 hours after the

administration of graded doses of furazolidone by crop tube.

As shown in fig.15, there was no species difference between chickens and ducklings in the potency of furazolidone as an MAO inhibitor in the liver, heart and brain. Although furazolidone inhibited MAO activity in the duodenum in the duckling more than in the chicken in this experiment, this observation seems of little significance as it was not confirmed in other experiments ($\bar{c}$ table 6 & table 7).

A comparison of the potency of furazolidone as an MAO inhibitor in the turkey poult and chicken (fig.16) showed no species difference in the duodenum, liver or brain. However, in the heart, furazolidone was more potent as an MAO inhibitor in the turkey poult than in chickens. This apparent species difference was not observed when furazolidone was given to the birds in the feed (0.04% w/w, 10 days) when cardiac MAO was inhibited to similar extent in the two species (table 4). Thus further experiments are required to determine whether or not this effect is repeatable.

Influence of neomycin on the inhibition of MAO by furazolidone in chickens and ducklings

Chickens (8 weeks old) were given neomycin, either 20 mg by intramuscular injection or 200 mg by crop tube twice daily for 5 days before the MAO estimation. Furazolidone (200 mg/kg) was administered by crop tube

to saline- and neomycin-treated birds 24 hours before the enzyme estimation. Neomycin did not affect MAO activity and the values obtained from chickens pretreated with saline or neomycin and given acacia mucilage (crop tube) were pooled and used as controls. As shown in table 6, furazolidone administered by crop tube inhibited MAO activity in each organ as before ($P < 0.001$), and pretreatment with neomycin by the intramuscular route did not affect the MAO inhibition ($P > 0.1$). On the other hand, pretreatment with neomycin by crop tube significantly reduced the MAO inhibiting action of furazolidone in each organ ($P < 0.01$ to < 0.001). Following oral administration of neomycin and furazolidone (by crop tube) the activity of MAO was less than the control value in the liver ($P < 0.002$) and brain ($P < 0.05$) but not in the duodenal mucosa ($P > 0.1$) and heart ($P > 0.05$). Thus the suppression of the MAO inhibiting action of furazolidone by the oral neomycin was complete in the duodenal mucosa and heart but not in the liver and brain.

In another experiment, furazolidone and neomycin were administered by crop tube to ducklings aged 2 - 8 days, to see whether or not neomycin would antagonize the action of furazolidone as an MAO inhibitor in this species. In neomycin-treated ducklings the furazolidone did not inhibit MAO activity in the liver ($P > 0.05$) and brain ($P > 0.1$) (table 7), although it did so in the controls ($P < 0.01$).

Effect of furazolidone on aminopyrine demethylase activity

In two experiments the effect of furazolidone on aminopyrine demethylase activity in a microsome-rich supernatant fraction of liver was investigated (table 8). In one experiment the drug was given in the feed at the therapeutic dose (0.04% w/w, 10 days) and in the other by crop tube at a dose of 200 mg/kg 24 hours before the estimation of the enzyme activity. In both experiments the activity of aminopyrine demethylase was unaffected by the drug.

Discussion

Furazolidone administered in the feed at a concentration of 0.04% w/w for 10 days inhibited MAO activity in the duodenal mucosa, heart and brain of chickens, ducklings and turkey poults. Only in ducklings was there a significant inhibition of the activity of MAO in the liver. MAO is inhibited by a transformation product of furazolidone (Stern et al, 1967; Pettinger et al, 1968) and MAO inhibition would be expected at the site of transformation. As MAO activity was not inhibited in the liver of chickens and turkey poults, it seems improbable that the transformation of furazolidone occurred here. The inhibition of MAO activity in liver of ducklings treated with furazolidone (0.04% w/w, 10 days) may indicate that the hepatic clearance of the active metabolite is less efficient in this species. The very low activity of mixed function oxidase in the duck liver (Bartlet & Kirinya, 1976) lends support to this

suggestion.

Following withdrawal of medication from chickens, the enzyme activity recovered more promptly in the duodenal mucosa than in other organs. The recovery of activity may be due to synthesis of new enzyme, as dialysis does not restore activity to MAO inhibited by furazolidone (Stern et al, 1967).

Furazolidone administered to chickens by crop tube inhibited the activity of MAO in the duodenal mucosa, liver, heart and brain. Thus, in the chicken, furazolidone given as a bolus inhibited the hepatic enzyme, although it did not do so when fed continuously. In the hepatocytes, the furazolidone metabolite may inhibit mitochondrial MAO, or it may be metabolized further and/or excreted with bile. The present observation suggests that the clearance of the metabolite was insufficient to protect hepatic MAO from inhibition when the drug was given as a bolus (200 mg/kg), but was sufficient when it was given as a feed additive (0.04% w/w, 10 days). In chickens, following furazolidone by crop tube, a maximum inhibition of MAO in the liver was found after 12 hours and in other organs after 24 hours. This observation suggests that the hepatic clearance of the furazolidone metabolite resulted in a briefer inhibition of MAO in the liver than elsewhere.

Furazolidone administered to chickens by intramuscular injection did not affect the activity of MAO in any of the organs studied. This observation suggests that conversion

of the drug to an MAO inhibitor did not occur in the bird's tissues. However, this finding may not extend to other species as furazolidone given by intravenous or intraperitoneal injection, is reported to inhibit MAO in the rat (Stern et al, 1967).

The alimentary flora might have produced the metabolite which inhibited MAO activity. To test this hypothesis, MAO activity was measured in different parts of the alimentary tract in chickens given furazolidone at a concentration of 0.04% w/w for 10 days. The inhibition of MAO activity was most marked in the rectal caeca and crop, where the alimentary flora is most abundant in the chicken (Jayne-Williams & Fuller, 1971). Further support for the hypothesis was obtained from the experiment in which furazolidone was given to chickens by crop tube after suppressing the alimentary flora with neomycin. Pretreatment with neomycin by crop tube antagonized the action of furazolidone on MAO activity, but neomycin injected intramuscularly at one-tenth of the oral dose did not do so. Less than 10% of neomycin is absorbed from the alimentary tract (Waksman, 1953; Freyburger & Johnson, 1956), so in these experiments absorbed neomycin could not have influenced the actions of furazolidone on MAO activity. The inhibition of MAO activity by furazolidone was suppressed by unabsorbed neomycin, which supports the hypothesis that the alimentary flora transform furazolidone to an active metabolite which is

subsequently responsible for the inhibition of MAO activities in other organs. In ducklings, the inhibition of MAO activity following the administration of furazolidone by crop tube was antagonized by neomycin given by the same route. This observation suggests that the alimentary microflora transformed furazolidone to an MAO inhibitor in ducklings, as in the chicken.

Furazolidone (administered by crop tube) was about equipotent in chickens, ducklings and turkey poults as an inhibitor of MAO activity. Thus in these species, the alimentary microflora seemed equally active in transforming furazolidone to an MAO inhibitor.

Many drugs inhibit both MAO and mixed function oxidase (Kato, Takanaka & Shoji, 1969). Aminopyrine demethylase activity in the chicken liver, however, was unaffected by furazolidone treatment (0.04% w/w, 10 days, or 200 mg/kg by crop tube). Thus furazolidone at the recommended therapeutic dose would not be expected to potentiate the actions of other drugs metabolized by mixed function oxidase.

Chapter II

The pharmacological consequences of MAO inhibition by furazolidone

Having established that the avian MAO activity was inhibited by furazolidone, the next consideration was whether or not it was sufficient to give rise to pharmacological effects. It is known that the inhibition of MAO activity may lead to an increase in the amounts of tissue monoamines (Spector, 1963), potentiate some of their actions (Goldberg & Sjoerdsma, 1959), and increase the urinary excretion of unmetabolized monoamines (Brunjes, Haywood & Maronde, 1963).

In the following experiments, the effect of furazolidone on the amounts of monoamines in the brain of chickens and ducklings, and on the vasopressor and vasodepressor actions of some amines have been investigated.

Results

The effect of furazolidone (0.04% w/w, 10 days) on the amounts of 5-HT, adrenaline and noradrenaline in the brain

Furazolidone was given in the feed to chicks and ducklings (aged 2 days at the commencement of the experiment) and the amounts of 5-HT, adrenaline and noradrenaline measured in the brains of control and treated birds. The results of this experiment (Table 9) show that following furazolidone treatment the amount of 5-HT in the brain was increased significantly in both chicks ($P < 0.001$) and ducklings ($P < 0.05$), whereas the amounts of adrenaline

and noradrenaline were unaffected.

The effect of furazolidone on the vasopressor actions of noradrenaline and tyramine

The regressions of the vasopressor responses upon dose for noradrenaline and tyramine injected intravenously in anaesthetized preparations of chickens are shown in fig. 17. The response to noradrenaline in preparations made from chickens given furazolidone in the feed (0.04% w/w, 10 days) were the same as those in control preparations. In contrast, pretreatment with furazolidone at the therapeutic dose potentiated the vasopressor action of tyramine 2.5 - 5 fold ($P < 0.01$).

Tyramine hydrochloride (5 mg/kg and above) injected intraduodenally in anaesthetized preparations of chickens produced a prolonged rise in arterial blood pressure, the response reaching a maximum in about 6 minutes and subsiding over 6 - 9 minutes (fig. 18). The vasopressor action of intraduodenal tyramine was potentiated about 5-fold in preparations made from chickens treated with furazolidone (0.04% w/w, 10 days) (Table 10), the action of 5 mg/kg of the drug in the furazolidone-treated preparations not being significantly different ($P > 0.1$) from 25 mg/kg in the controls.

The effect of furazolidone (0.04% w/w, 10 days) on the vasodepressor actions of 5-HT, histamine and tryptamine

5-HT, histamine and tryptamine were injected intra-

venously into anaesthetized preparations of control and furazolidone-treated chickens, and the vasodepressor responses recorded. There was no significant difference between the vasodepressor actions of the amines in the control and the drug-treated preparations (fig. 19) ($P > 0.1$).

Discussion

Furazolidone, given in the feed at the therapeutic dose for galliformes increased the amount of 5-HT in the brains of chickens and ducklings. The increase in 5-HT was selective, as the amounts of adrenaline and noradrenaline in the brains were unaffected by the treatment. This finding is not atypical, e.g. administration of an MAO inhibitor to the rabbit may produce a selective increase in the amount of 5-HT in the brain (Spector, Shore & Brodie, 1960). In all probability treatment with furazolidone at a higher dose would have increased the amount of catecholamines in avian brain. Indeed, the administration of furazolidone (500 mg/kg) to the rat produced an increase of both 5-HT and noradrenaline in the brain (Palm, Magnus, Grobecker & Jonsson, 1967). The behavioural stimulation noted in ducks and pigs given furazolidone (Lucas, 1956; Borland, 1979), was most probably due to the increased amount of monoamines in the brains of the affected animals.

Furazolidone given in the feed potentiated the vasopressor action of tyramine administered intravenously.

In vivo, tyramine is metabolized by oxidative deamination whereas noradrenaline is mostly inactivated by other routes, and the present findings indicate that following furazolidone therapy the inhibition of MAO activity in the vasculature and the heart was sufficient to potentiate the action of tyramine in the systemic circulation.

Tyramine (5 mg/kg and above) injected into the duodenum produced a vasopressor response in control preparations, indicating that a significant amount of the amine was absorbed. Pretreatment of the birds with furazolidone (0.04% w/w, 10 days) potentiated the vasopressor action of tyramine about five-fold, regardless of whether the amine was given intravenously or intraduodenally. Thus in the chicken, furazolidone at the therapeutic dose seemed to have little influence on the absorption of tyramine into the systemic circulation from the gut. Although more of the tyramine may have been absorbed into the portal circulation from the duodenum when the MAO activity in the alimentary tract was inhibited, it seems that the amount reaching the systemic circulation was not affected significantly, probably because the hepatic MAO was not inhibited in the furazolidone-treated preparations.

The vasodepressor actions of histamine, 5-HT and tryptamine were not significantly affected by the furazolidone treatment. The reason for this is not clear, but the drug is known to inhibit the activities of both MAO and diamine oxidase (DAO) (Palm & Magnus, 1968), and in the

present experiments, it seems that the inhibition of these enzymes in the heart, blood and vasculature was not sufficient to potentiate the actions of the injected amines. An alternative explanation for the lack of potentiation of histamine and 5-HT is that histamine is only partly metabolized by DAO and that its fate depends above all on the activity of imidazole-N-methyl transferase. Although injected 5-HT is mostly metabolized by MAO, a considerable proportion of the endogenous amine is cleared by conjugation with glucoronic acid (Bartlet, 1971), and it is possible that following MAO inhibition by furazolidone, 5-HT was cleared by conjugation rather than oxidative deamination.

Chapter III

Anorexia in poultry given furazolidone

A marked reduction in the growth of birds treated with furazolidone (0.04% w/w, 10 days), was noted in the previous experiments, and the nature of the phenomenon is examined here.

In the description of the following experiments the word "furazolidone" indicates that the drug was given in the feed at a concentration of 0.04% w/w for 10 days, and the age of the birds on the first day of the feeding is given in parenthesis.

Results

The effect of furazolidone on the feed intake and growth of poultry kept in groups (3-4 birds)

The furazolidone was given to chickens (7½ weeks) and the feed intake and growth measured daily. In the treated birds the feed intake was significantly less than that in the controls ($P < 0.05$) (fig.20), and the growth (with respect to the initial body weight and feed consumption) was also reduced ($P < 0.001 - < 0.002$). The feed intake and growth in four groups of ducklings (4 days) were similarly inhibited by furazolidone ($P < 0.05 - < 0.02$) (fig. 20.

In one group of turkey poults (4 days) furazolidone reduced the feed intake to 2.1 (kg)/kg body weight/10 days, while the control group consumed 2.9 (kg)/kg body weight/ 10 days. The growth of the treated birds, 754.5 ± 90.2 (g)/kg body weight/10 days (n = 6 birds), was less than that

in the controls, 1310.3 ± 101.2 (g)/kg body weight/10 days (n = 6) ($P < 0.01$).

The feed intake and growth in chickens housed individually and fed ad libitum

In order to proceed to a pair-feeding experiment, it was necessary to obtain data on the feed intake and growth of birds housed individually. It was noted that the feed intake and growth of the individually-housed chickens was less than that in the grouped birds. This is in accord with other workers' findings (Tolman, 1964; Tolman & Wilson, 1965) that a chicken eats more in the presence of a companion than in isolation.

Chickens (7½ weeks) were caged singly and given furazolidone, the controls receiving an unmedicated diet. In the treated birds growth was less than in the controls ($P < 0.01$) on days 6 - 10 of the experiment (fig.21). No significant difference, however, was found between the cumulative feed intakes of the control and treated birds.

Growth in pair-fed chickens

Chickens of the same age as those used in the previous experiment were housed individually and their growth recorded. The birds were divided into two groups and given restricted diets of unmedicated food, the amount being equivalent to that consumed ad libitum by the control and the furazolidone-treated birds in the previous experiment.

The two groups of pair-fed chickens grew at about the same rate on days 1 - 4 of the experiment ($P > 0.05 - 0.1$)

(fig. 22). However, on days 5 to 10, the chickens pair-fed with furazolidone-treated birds grew significantly less than those pair-fed with control birds ($P < 0.05 - <0.02$).

The growth of the pair-fed birds is compared with that of those fed ad libitum in fig. 23. There was no significant difference in growth between the birds fed ad libitum with control diet and their pair-fed counterparts ($P > 0.1$) (fig.23A). Moreover, the growth of chickens fed ad libitum with furazolidone was not significantly different ($P > 0.1$) from that in pair-fed birds on control diet (fig.23B). However, the pair-fed birds showed a tendency to grow more than the drug-treated birds on days 7 to 10 of the experiment, although this did not reach significance.

The effect of methergoline on the feed intake and growth of chickens

This experiment examined the effect of methergoline, a potent and long-lasting antagonist of the central actions of 5-HT (Beretta, Ferrini & Glässer, 1965; Mawson & Whittington, 1970), on the feed intake and growth of chickens (7½ weeks) kept in groups and given furazolidone. The methergoline was dissolved in 2% w/v aqueous ascorbic acid and injected intramuscularly at a dose of 1 mg/kg twice daily for the 10 days of the experiment.

Methergoline produced no significant improvement in the feed intake or the growth of chickens ($P > 0.1$) (table 11).

Discussion

The influence of furazolidone on the growth of chickens was studied as early as 1953, when it was shown that feeding the drug at a low concentration (0.006% w/w for 10 weeks) did not affect the growth of chickens kept under hygienic conditions (Jacobs et al, 1953). Thereafter, several workers (Cooper & Skulski, 1956; McDonald & Beilharz, 1961; Jensen et al, 1975; Ehlhardt, 1975) found that growth in chickens was reduced when furazolidone (0.033% - 0.04% w/w) was given for one to four weeks. However, in most of these studies the feed intake of the birds was not measured, and the reduction in growth produced by the drug was attributed variously to impaired digestion, absorption or metabolism.

In the present experiments, the reduction in feed intake in the furazolidone-treated birds did not result from a distaste for the drug, as it did not start on the first day of the treatment but took some days to develop.

The growth of the chickens fed ad libitum with furazolidone was not significantly different from that of the birds pair-fed with unmedicated food. This observation suggests that, in the furazolidone-treated chickens, the reduction in growth was mainly due to the reduced feed intake. This conclusion is supported by the significant difference in growth between the chickens pair-fed with the furazolidone-treated and the control birds. Thus the reduction in growth in the furazolidone-treated birds could be fully accounted for by the anorexia.

It seemed that the increase in brain 5-HT in the furazolidone-treated birds might have given rise to the anorexia, as 5-HT is known to play a role in the regulation of feed intake (Blundell, 1977; Blundell & Latham, 1978). In the rat, methergoline (1 mg/kg; i.p.) antagonized the anorectic action of m-chloro-phenylpiperazine (an agonist at the central tryptaminergic receptors) (Samanin, Mennini, Ferraris, Bendotti, Borsini & Grattis, 1979). In the present experiments, however, methergoline (1 mg/kg, intramuscularly twice daily) did not antagonize the anorexia significantly in the furazolidone-treated chickens. Thus it seems unlikely that the anorexia in the birds was an outcome of the increase in brain 5-HT.

Chapter IV

Effect of furazolidone on the thiamin status of birds

In the rat, the monoamine oxidase (MAO) inhibitors iproniazid, harmaline and phenylcyclopropylamine increased the concentrations of pyruvate and lactate in blood (Gey & Pletscher, 1961). This effect was attributed to an inhibition of the metabolism of monoamines, because it occurred simultaneously with an accumulation of 5-HT in the brain, and because 5-HT and noradrenaline given subcutaneously increased pyruvate and lactate in blood. However, doubt is cast upon the above explanation by the observation in man that nitrofurazone, which does not inhibit MAO (Quiring & Palm, 1970), also produced an increase in blood pyruvate (Knight, 1959; Robertson, 1959).

In the turkey, the concentrations of pyruvate and lactate in blood were increased after treatment with furazolidone (0.04% w/w, 28 days) (Staley et al, 1978), and the drug produced a cardiomyopathy which resembled that occurring in thiamin deficiency in man (beriberi). A rise in blood pyruvate and lactate can also be a sign of thiamin deficiency (Horwitt & Kreisler, 1949).

In the present experiments furazolidone produced anorexia, another common sign of thiamin deficiency. Thus it seemed of interest to determine the thiamin status of birds treated with furazolidone. In all the

following experiments the drug was given in the feed at a concentration of 0.04% w/w for 10 days, and the age of the birds on the 10th day of the feeding is given in parenthesis.

Results

Effect of furazolidone on the concentrations of pyruvate and lactate in the blood of poultry

The concentrations of pyruvate and lactate were measured in the blood of chickens (9 weeks), ducklings (2 weeks) and turkey poults (2 weeks) given furazolidone. The drug produced a significant increase ($P < 0.05$-<0.001) in the concentrations of pyruvate and lactate in the blood of the three species (fig. 24).

Influence of thiamin on the concentrations of pyruvate and lactate in furazolidone-treated chickens

In this experiment with chickens (9 weeks), thiamin hydrochloride was injected intramuscularly at a dose of 84 µg/kg (twice daily, at 9.00 a.m. and 4.45 p.m.) during the administration of furazolidone. The dose of thiamin was slightly above the daily requirement of this vitamin for 7½ weeks old chickens of light breed (see table 11 in Nutrient Requirements of Poultry, Anonymous, 1971). The thiamin did not produce a significant reduction in the concentrations of pyruvate and lactate in the blood of chickens given furazolidone ($P > 0.05$) (table 12). Moreover, furazolidone still

raised the concentration of pyruvate in chickens injected with thiamin ($P < 0.01$), although the lactate concentration was not increased significantly ($P > 0.05$).

Effect of furazolidone on the activity of transketolase (TK) and the thiamin pyrophosphate (TPP) effect in chicken haemolysates

The activity of TK in the haemolysates was not significantly affected following administration of furazolidone to the chicken. On the other hand, the increase in TK activity produced upon addition of TPP to the haemolysates (TPP effect) was significantly greater in preparations from furazolidone-fed chickens than in controls ($P < 0.02$) (fig. 25).

Influence of thiamin on TK activity and the TPP effect in chickens given furazolidone

Although thiamin given intramuscularly at the above dose did not affect the TK activity in either furazolidone-treated or control chickens ($P > 0.1$), it produced a significant decrease ($P < 0.05$) in the TPP effect in chickens given furazolidone (table 13). The thiamin did not reduce the TPP effect to the normal value, however, as it was still higher in birds treated with furazolidone and thiamin than in those given thiamin only ($P < 0.001$).

Effect of thiamin on the anorexia produced by furazolidone

In one experiment, the chickens were 8 weeks old at the start of feeding, and in another 11 weeks. Because

of the difference in feed intake and growth of the birds at these ages, the data from the two experiments are presented separately (table 14). The growth of the control and the furazolidone-treated chickens was not significantly affected ($P > 0.1$) by thiamin, nor was their feed intake.

Effect of furazolidone on oxygen consumption of chicken tissues

The oxygen consumption of the duodenal mucosa, liver, heart and brain from control and furazolidone-fed chickens (9 weeks) has been measured (fig. 26). In the homogenates of heart prepared from drug-treated birds, the oxygen consumption was significantly greater than that in control preparations ($P < 0.02$ for 200 and 400 mg tissue). However, furazolidone did not produce any significant change in the oxygen consumption of the other organs, with the exception of liver (400 mg) where it was reduced ($P < 0.001$).

Effect of thiamin on MAO activity in chickens treated with furazolidone

In the chicken, thiamin did not affect the MAO inhibitory action of furazolidone in the caecal mucosa, heart and brain ($P > 0.1$) (table 15).

Discussion

In thiamin deficiency, the concentrations of pyruvate and lactate are increased in the blood (Horwitt & Kreisler, 1949), and in haemolysates there is a decrease in the TK activity and an increase in the TPP effect (Brin, 1962; Brin, Tai, Ostashever & Kalinsky, 1960; Sauberlich, 1967). According to Brin (1974) a TPP effect of 15-24% suggests a borderline deficiency of thiamin, and an acute thiamin deficiency (usually with clinical signs) is indicated by a TPP effect greater than 25%. These biochemical indices were useful in assessing the thiamin status of chickens given furazolidone.

In the present experiments, furazolidone increased the TPP effect and the concentrations of pyruvate and lactate in blood, and produced anorexia. The magnitude of the TPP effect in the furazolidone-treated chickens suggested that the drug had produced a borderline deficiency of thiamin. Although only anorexia and blood pyruvate and lactate concentrations were measured in turkey poults and ducklings, the drug seemed to produce a similar effect in these species.

Thiamin was given to furazolidone-treated chickens to try to reverse the signs of its possible deficiency (the anorexia, the increased concentrations of blood pyruvate and lactate, and increased TPP effect). The vitamin did not counteract significantly either the anorexia or the increase in blood pyruvate and lactate

concentrations, although it produced some reduction in the TPP effect. It should be mentioned that in the chicken, thiamin at 2/3rd the present dose was effective in abolishing the adverse effects of amprolium, a known thiamin antagonist (Polin, Wynosky & Porter, 1962). Thus in the present experiments, the failure of thiamin to counteract the signs of its possible deficiency seems to suggest that furazolidone may have interfered with its utilization.

There are, however, some observations that seem inconsistent with the proposition that furazolidone induced a thiamin deficiency in the birds. It is known that the oxygen consumption of brain tissue from thiamin-deficient pigeons is reduced (Banga, Ochoa & Peters, 1939). Yet, in the present experiments, treatment of chickens with furazolidone did not affect the oxygen consumption of brain homogenates. Indeed, a significant increase was found in the oxygen consumption of the heart following the administration of furazolidone. This phenomenon is not understood, but could be due to the accumulation of pyruvate and lactate, as these substrates are known to be an important source of energy in the heart (Bing, 1955; Opie, 1969). The increase in the hepatic and cardiac glycogen concentrations in turkeys given furazolidone (Czarnecki et al, 1974) also seems inexplicable in terms of thiamin deficiency.

The MAO inhibitory action of furazolidone was

unaffected by thiamin. As the dose of the vitamin was ineffective in reversing signs of its possible deficiency, one cannot reach a conclusion as to whether or not the signs of thiamin deficiency and the inhibition of MAO were related phenomena. It has been reported, however, that MAO activity in thiamin-deficient rats was lower than in controls, and that it could be restored to a normal value by repeated injections of thiamin (Iwata, Nishkawa & Fujimoto, 1969).

Chapter V

Effect of Furazolidone on the Adrenal Glands of the Chicken

According to Palm, Magnus, Grobecker & Johnsson (1967), administration of furazolidone (500 mg/Kg, orally) to the rat reduced the amount of catecholamines in the adrenal medulla by about 30%. In the chicken, furazolidone (given in the feed at a concentration of 0.04% w/w for one week or 0.01% w/w three weeks) has been reported to increase the level of corticosterone* in plasma (Sidorov & Lüders, 1971) and it was suggested that the drug produced a non-specific "stressor" effect.

An apparent increase in the weight of the adrenal gland in chickens, treated with furazolidone (0.04% w/w, 10 days) led us to examine the effect of the drug on this organ. The amount of catecholamines in the adrenal medulla and several parameters of adreno-cortical function were measured. In all the following experiments furazolidone was given to the chickens in the feed at a concentration of 0.04% w/w for 10 days.

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* It should be mentioned that the estimation of corticosterone in this report is of dubious validity, as the fluorimetric method used was shown subsequently to be neither specific nor accurate in measuring avian corticosterone (Freeman, Manning & Flack, 1979).

Results

Influence of furazolidone on the catecholamines in the adrenal glands

Adrenaline and nor-adrenaline were measured in the adrenal glands of control and furazolidone-treated cockerels. The drug did not affect the amount or the concentration of either amine in the adrenal glands ($P > 0.1$ in each instance, table 16).

Effect of furazolidone on concentrations of ascorbic acid and cholestrol in the adrenal glands, and cholestrol and glucose in plasma

Treatment of cockerels with furazolidone produced a small and statistically insignificant ($P > 0.05$) reduction in the concentration of cholestrol in the adrenal glands. The drug had no effect on the concentrations of ascorbic acid in the adrenal glands, and cholestrol and glucose in plasma ($P > 0.1$ in each instance, table 17).

Effect of furazolidone on organ weights

The weights of the adrenal glands, heart, pancreas and thyroid glands were compared in control and furazolidone-treated cockerels (table 18). Only the adrenal glands of the treated birds showed a tendency to increase in weight (g), and this increase did not reach significance ($P > 0.1$). However, when the organ weights were expressed as percentrages of body weight, significant increases were found

in the values for the adrenal ($P < 0.05$) and the thyroid gland ($P < 0.1$) in the treated birds. The weights of the heart and the pancreas (expressed as a percentage of body weight) were not significantly affected by the drug ($P > 0.1$ in each instance).

Discussion

The amount of catecholamines in the adrenal glands was not affected by furazolidone. Thus, any effect of the drug on adrenal weight would most probably be due to an effect on the cortex.

As the furazolidone-treated chickens grew less than the controls, one would expect the organ weights of the treated birds to be smaller than those of the controls. However, the adrenal glands in chickens given furazolidone tended to be larger than those in controls. This effect on the adrenal weight was selective as the weights of the pancreas, heart and thyroid glands (g) were not increased by the treatment. The influence of the drug on the weights of the adrenal and thyroid glands attained significance when the weights were expressed as percentages of body weight, both glands being enlarged in the treated birds.

Furazolidone produced no significant change in the concentrations of ascorbic acid and cholesterol in the adrenal glands or in the concentrations of cholesterol and glucose in the plasma. Thus, the present findings neither confirm nor refute the suggestion of Sidorov & Lüders (1971) that furazolidone is a "stressor" agent in the chicken.

Experiments to determine the more sensitive indicators of "stress" (viz, plasma corticosterone and differential leucocyte count) are still required.

The furazolidone treatment produced a significant increase in the weight of the thyroid glands when expressed as a percentage of body weight. The implication of this observation is not clear, although some of the known effects of the thyroid hormones seem to concur with effects produced by furazolidone in the chicken (e.g. the decrease in growth and the increase in oxygen consumption of heart tissue).

Chapter VI

Some Untoward Effects of Furazolidone in Chickens

Furazolidone at doses above that used in therapy produced some untoward effects in poultry. For example, the drug has been reported to produce an anaemia in the chicken when fed at a concentration of 0.04% w/w for 21 days (Ehlhardt et al, 1975). In the turkey, furazolidone (0.05% w/w) in the feed for 28 days increased aspartate transaminase activity in plasma (Staley et al, 1978), and at a concentration of 0.05 - 0.07% w/w for 42 days, it decreased the arterial blood pressure (Simpson et al, 1979).

The present experiments examine whether or not the therapeutic dose of furazolidone (0.04% w/w, 10 days) produces these effects in chickens.

Results

Effect of furazolidone on arterial blood pressure in conscious chickens

The arterial blood pressure was measured in conscious chickens which had been given a control or a furazolidone-medicated diet. The blood pressure of birds given furazolidone was not significantly different ($P > 0.1$) from that of the controls (table 19).

Effect of furazolidone on packed cell volume (PCV) and haemoglobin (Hb) content of blood

The PCV and Hb content in the blood of chickens treated with furazolidone were not significantly different from that in controls ($P > 0.1$) (table 19).

Effect of furazolidone on the activity of aspartate transaminase in plasma

As shown in table 19, the furazolidone did not affect the activity of the enzyme in chicken plasma ($P > 0.1$).

Discussion

Treatment with furazolidone at the therapeutic dose produced a small reduction in the arterial blood pressure of the chicken, which was not statistically significant. The drug treatment also had no effect on the PCV or the Hb content of the blood, indicating that, at the therapeutic dose, the drug did not produce anaemia in the chicken.

Furazolidone did not affect the activity of aspartate transaminase in the plasma. This is a cytoplasmic enzyme, and the present observation suggests that furazolidone did not produce a detectable damage in cell membranes.

Thus, it is clear that, in the chicken, the therapeutic dose of furazolidone does not produce the untoward effects which have been reported in galliformes following administration of the drug at greater doses.

General Discussion

Furazolidone has found many applications as an antimicrobial agent in both man and animals. Nonetheless, the pharmacological effects of the drug on the host have received insufficient attention, and little work has been done to find out the 'biochemical lesions' which precede the manifestation of the toxic signs produced by the drug.

One established pharmacological effect of furazolidone in rat and man is the inhibition of MAO activity *in vivo* (Stern et al, 1967; Palm et al, 1967; Pettinger et al, 1968). Although furazolidone is not itself a MAO inhibitor, it is believed to be converted to an active metabolite, possibly 2-hydroxyethyl hydrazine, in the rat (Stern et al, 1967). However, Pettinger et al (1968) failed to detect any metabolite which possessed MAO inhibiting properties in the urine from patients treated with furazolidone. The exact site where transformation of furazolidone to the active metabolite takes place is not certain. The present experiments (Chapter 1) have indicated that the alimentary flora is the site of transformation. This was based on the finding that the intramuscular administration of furazolidone to the chicken was ineffective in inhibiting MAO activity, whereas oral furazolidone inhibited the enzyme. To verify the hypothesis that furazolidone was transformed by the alimentary flora, two sets of experiments were performed. Firstly, MAO activity was measured along the alimentary tract in the furazolidone-treated chickens.

It is known that the flora in the alimentary tract is most abundant in the caecum and the crop (Jayne-Williams & Fuller, 1971), and, if the hypothesis was valid, greater inhibition of MAO would be expected in the parts richest in the flora. This was indeed the case. Secondly, an attempt was made to suppress the alimentary flora by oral administration of neomycin. This antibiotic was chosen because of its broad spectrum activity and poor absorption from the gut. These experiments showed that oral neomycin did antagonize the MAO inhibitory action of furazolidone. It appears that less than 10% of oral neomycin is absorbed from the alimentary tract, at least in dogs and rats (Waksman, 1953; Freyburger & Johnson, 1956). When the antibiotic was given intramuscularly at one-tenth of the oral dose, it did not antagonize the inhibitory action of furazolidone indicating that absorbed neomycin could not have influenced the action of furazolidone on MAO activity. In ducklings, oral neomycin also antagonized the MAO inhibitory action of furazolidone, suggesting that the alimentary flora of ducklings transformed furazolidone to an inhibitor of MAO as in the chicken.

Although the role of the alimentary flora in transforming furazolidone has been indicated in more than one experiment, the ultimate proof for its involvement in transforming furazolidone to an MAO inhibitor would be the use of germ-free birds, but these were not available.

In the absence of germ-free birds, the results obtained with neomycin are worth confirming with another antibiotic, e.g. oxytetracycline. Our contention that furazolidone becomes an MAO inhibitor only after transformation by the alimentary flora has recently been confirmed in the rat (Cohen, 1980), using the antibiotic oxytetracycline to suppress the gut flora. It has been realized in recent years that a number of compounds containing nitro-group undergo metabolic transformation (mainly reductive) by the action of the alimentary flora (see e.g. Goldman, 1978; Facchini & Griffith, 1980).

It was of interest to note that furazolidone (0.04% w/w, 10 days) inhibited MAO activity in the liver of ducklings and not chickens, indicating that hepatic clearance of the inhibitor was less efficient in the former species than in the latter.

No mortality was found among ducklings given furazolidone in the feed at a concentration of 0.04% w/w for 10 days, or by crop tube at a dose of 200 mg/kg. These observations are at variance with reports of a high susceptibility to furazolidone in ducks (Dougherty, 1956; Kriz, Branda & Svestka, 1968). The toxicity of the drug in ducks seems variable, and it is not difficult to account for this now it is understood that the drug is transformed to an active metabolite by the alimentary flora. Free range conditions may encourage the proliferation of these components of the flora which transform the

drug to an active metabolite, and under these free range conditions, the ducklings may be more susceptible to the drug than in the present experiments.

A significant rise was found in the amount of 5-HT in the brain of chickens and ducklings following furazolidone treatment (0.04% w/w, 10 days) (Chapter 2). The amounts of noradrenaline and adrenaline were unaffected by the drug. The increase in 5-HT is most probably an outcome of MAO inhibition. The rise in brain monoamines was possibly involved in the nervous signs observed in ducks and pigs following furazolidone treatment (Lucas, 1956; Borland, 1979).

Furazolidone (0.04% w/w, 10 days) potentiated (to a similar extent) the vasopressor action of tyramine whether given intravenously or intraduodenally. The inhibition of MAO in the alimentary tract would be expected to interfere with the detoxification of pharmacologically active amines present in the diet or formed from the dietary amino acids in the intestine by bacterial decarboxylases. However, although more of the tyramine may have been absorbed into the portal circulation from the duodenum when MAO in the alimentary tract was inhibited, it seems that the amount reaching the systemic circulation was not affected significantly, probably because the MAO activity in the liver was not inhibited by this treatment in the chicken. Another possible experiment, which might be carried out to demonstrate the

consequences of MAO inhibition in the chicken, would be to test the effects of tyramine and noradrenaline of the rate and amplitude of isolated auricles from furazolidone-treated birds (Lock, 1963).

The addition of furazolidone to the diet (0.04% w/w, 10 days), was shown to produce a significant reduction in feed intake and growth of the treated birds (Chapter 3). The influence of the drug on growth has been reported by several authors before, but little or no attention has been paid to the effect of the drug on feed intake. In the present experiments an attempt was made to correlate the growth reduction with feed intake. The pair-feeding experiment showed that the growth of chickens fed ad libitum with control diet was matched very closely by that in pair-fed birds, indicating the validity of the technique of pair-feeding. Growth in chickens fed ad libitum with furazolidone (0.04% w/w) was not significantly different from that in birds pair-fed with unmedicated feed. Thus, the reduction in growth in the furazolidone-treated birds could be accounted for by the reduction in feed intake. However, a tendency of the furazolidone-treated birds to grow less than pair-fed birds must not be overlooked, as it may be of biological significance.

An explanation for the anorectic action of furazolidone was sought in the increase in the 5-HT content of the brain. The feed intake and growth of chickens medicated concomitantly with furazolidone and methergoline (a potent antagonist of central actions of 5-HT) were not different

from those in chickens given furazolidone only. Thus the involvement of the rise in 5-HT in the anorectic action of furazolidone seemed unlikely.

The commonly used indices of thiamin status were employed in the present experiments to investigate the possibility that furazolidone had influenced the thiamin status of the birds (Chapter 4). The measurement of the activities of enzymes in which TPP is a cofactor is the best parameter for determining the thiamin status. These enzymes include transketolase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. The effect of thiamin deficiency on the activity of transketolase is particularly pronounced, and in the present experiments the activity of this enzyme and its stimulation by TPP *in vitro* (TPP effect) has been measured in the blood. An increase in the concentrations of pyruvate and lactate in the blood is also considered an indicator of thiamin deficiency because the decarboxylation of pyruvate to acetyl coA may be limited when TPP is deficient.

Furazolidone treatment raised the blood concentrations of pyruvate and lactate, and increased the TPP effect on transketolase activity. It did not, however, affect significantly the basal transketolase activity in the erythrocytes. The drug also produced a reduction in the feed intake and growth of the treated birds. Taken together, these findings seem to indicate that the treatment had produced a degree of thiamin deficiency in

the birds. It is possible, however, that these biochemical effects could have arisen indirectly from inanition rather than from the drug per se. Measuring the indices of thiamin status in pair-fed chickens would help to discriminate between these possibilities.

The attempt to reverse the furazolidone-induced signs of thiamin deficiency by injecting the birds with thiamin HCl was unsuccessful. It is unlikely that the dosage of the vitamin was insufficient, as a lower dose of thiamin was effective in reversing the signs of thiamin deficiency produced by amprolium (Polin et al, 1962). Furazolidone could have interfered with the utilization of the vitamin by the birds, and to test this possibility, the phosphorylated form of thiamin (TPP) will be given to the drug-treated chickens in further experiments. However, these experiments may be inconclusive as one cannot administer TPP intracellularly. Other enzymes whose activity might be studied to verify the effect of furazolidone on the thiamin status of the birds are pyruvate dehydrogenase (which catalyzes the transformation of pyruvate to acetyl coA in the mitochondria) and possibly thiamin phosphokinase (which catalyzes the phosphorylation of the thiamin in the cytoplasm).

The proposition of Sidorov & Lüders (1971) that furazolidone is a 'stressor' agent in the chicken was tested by measuring several indicators of 'stress'. However, the results obtained were inconclusive (Chapter 5).

There was an increase in the weight of the adrenal glands (about 20%), and a trend towards depletion of cholesterol from the gland which did not reach significance. The indicators of 'stress' used in these experiments (which are commonly used in mammals) have been shown to be applicable to the fowl (Freeman, 1970; Siegel, 1970). Other changes, mostly resulting from the increase in adrenal activity, can also be used as indicators of 'stress'. These include an elevation of plasma corticosterone, involution of lymphoid tissue (the thymus, the spleen and particularly the bursa of fabricus), and changes in the number of leucocytes. More experiments are required to elucidate the position of furazolidone as a 'stressor' agent in the chicken.

Furazolidone given to the chicken at the therapeutic dose did not produce the untoward effects which have been reported in poultry given the drug at larger doses (Chapter 6). Thus it seems that the toxicity is dosage and time-dependent. This was reassuring, indicating that the drug was safer than might have been supposed from the previously published work.

From a practical point of view, the reduction in feed intake and growth is probably the most serious effect of furazolidone in the birds. It seems unlikely, however, that this effect would be as great as the inhibition in growth which would result from an unmedicated infection.

Table 1.

The antimicrobial effect of furazolidone in poultry

Species	Disease or organisms	Drug concentration in the feed	Reference
Chickens	Salmonellosis a) *Salmonella gallinarium* b) *Salmonella typhimurium* c) *Salmonella pullorum*	0.02-0.04% w/w for 10 days	Smith (1954,1955)
	Escherichia coli	0.04% w/w for 10 days	Veterinary Data Sheets (1980)
	Coccidiosis a) *Eimeria tenella* & *Eimeria necatrix*	0.005%-0.011% w/w for 6-15 days	Harwood, Stunz & Wolfgang (1958)
	b) *Eimeria necatrix*	0.0165%-0.055% w/w for 10 days	Wolfgang, Stunz & Harwood (1957)
	Synovitis	0.011% w/w continuously (for prevention) 0.017% w/w for 7-10 days (for treatment)	Cosgrove (1957)
Turkey	*Salmonella gallinarium*	0.005% w/w for 14 days	Grumbles, Wills & Boney (1954)
	Histomonas meleagridis	0.011% w/w continuously (for prevention) 0.017% w/w for 7 days (for treatment)	Horton-Smith & Long (1956)
	Hexamita meleagridis	0.011% w/w continuously (for prevention) 0.011% w/w for 14 days (for treatment)	Mangrum, Ferguson & Crouch (1955)
Duck	*Salmonella anatum* *Salmonella typhimirium*	0.011% w/w for 14 days	Lucas (1956)

Table 2 .The percentage recovery of noradrenaline and 5-HT from the CG50 resin columns, the total extraction procedure and from chicken brain homogenates

	5-HT	noradrenaline
% recovery of amine (5 µg) passed through CG50 resin columns	93.1 ± 2.1 (5)	81.8 ± 1.4 (5)
% recovery of amine (5 µg) after total extraction procedure	89.8 ± 0.7 (5)	78.4 ± 1.8 (5)
% recovery of amine (1 µg) added to brain homogenate	81.0 ± 2.0 (24)	104.0 ± 5.0 (24)

The values in the table are means ± s.e.m. (number of observations)

For statistical comparisons see text.

Table 3. Monoamine oxidase (MAO) activity in homogenates of organs from chickens of different ages.

	MAO activity (µmol 4-hydroxyquinoline/g tissue/h)		
	1 day	8 weeks	P
duodenum	36.9 ± 5.1 (6)	90.7 ± 12.5 (8)	<0.02
liver	2.5 ± 0.4 (6)	22.3 ± 1.9 (7)	<0.001
heart	1.2 ± 0.1 (6)	3.3 ± 0.3 (7)	<0.001
brain	12.7 ± 1.1 (7)	15.6 ± 2.0 (7)	>0.2

The values in the table are means ± s.e.means (No. of chickens)

Table 4. Comparison of monoamine oxidase (MAO) activities in tissue homogenates from chickens, ducklings and turkey poults given furazolidone (0.04% w/w) in the feed for 10 days

	MAO activity, % control				
	chickens (1)	ducklings (2)	turkey poults (3)	P ($\bar{c}$f 1&2)	P ($\bar{c}$f 1&3)
duodenum	58.0 ± 1.7 (3)	59.4 ± 7.7 (4)	62.0 ± 6.9 (5)	>0.1	>0.1
liver	96.1 ± 2.1 (3)	79.6 ± 6.1 (4)	93.6 ± 11.1 (5)	<0.05	>0.1
heart	59.6 ± 10.6 (3)	55.2 ± 2.7 (4)	41.7 ± 5.1 (5)	>0.1	>0.1
brain	65.0 ± 8.6 (3)	64.5 ± 4.1 (4)	62.0 ± 14.9 (5)	>0.1	>0.1

The values in the table are means ± s.e. means (no. of animals)

Table 5. Effect of time on monoamine oxidase (MAO) inhibition in the chicken produced by furazolidone (200 mg/kg) given by crop tube.

Time (h) after furazolidone	MAO activity (μmol 4-hydroxyquinoline/g tissue/h) duodenal mucosa	liver	heart	brain
0	95.95 ± 6.21 (3)	29.72 ± 4.70 (3)	2.51 ± 0.21 (3)	15.41 ± 1.76 (3)
12	40.43 ± 0.92 (3)	9.93 ± 0.31 (3)	0.90 ± 0.01 (3)	8.80 ± 0.41 (3)
24	34.66 ± 1.67 (3)	11.65 ± 0.53 (3)	0.92 ± 0.10 (3)	7.02 ± 0.85 (3)
48	36.63 ± 2.65 (3)	15.27 ± 1.12 (3)	0.82 ± 0.06 (3)	8.04 ± 0.18 (3)

The values in the table are means ± s.e. means (No. of chickens).

Table 6. Monoamine oxidase (MAO) activity in homogenates of chicken organs 24 hours after administration of furazolidone (200 mg/kg, crop tube) and the influence of neomycin thereon

	MAO activity (4-Hydroxyquinoline, μ mol / g tissue / h)					
	(1) control	(2) Furazolidone	(3) Furazolidone + *neomycin (i.m.)	(4) Furazolidone + **neomycin (oral)	P Intramuscular neomycin [cf. (3) and (2)]	P oral neomycin [cf. (4) and (2)]
duodenal mucosa	93.71±5.29 (15)	32.83±6.65 (6)	38.10±2.20 (3)	75.19±11.54 (6)	>0.1	<0.01
liver	24.88±1.90 (15)	8.95±1.26 (6)	9.69±0.25 (3)	15.15±1.38 (6)	>0.1	<0.01
heart	2.66±0.13 (15)	0.81±0.09 (6)	1.18±0.30 (3)	2.32±0.14 (6)	>0.1	<0.001
brain	11.77±0.71 (15)	7.11±0.65 (6)	7.38±0.37 (3)	10.05±0.27 (6)	>0.1	<0.002

The values in the table are means ± s.e. means (No. of animals).

* Neomycin, 20 mg/bird by intramuscular injection, twice daily for 5 days before the MAO estimation.

** Neomycin, 200 mg/bird by crop tube, twice daily for 5 days before the MAO estimation.

Table 7. Monoamine oxidase activity in homogenates of duckling organs 24 hours after the administration of furazolidone (200 mg/kg, crop tube) and the influence of neomycin thereon.

	Monoamine oxidase activity (μ mol 4-hydroxyquinoline/g/h)						
	(1) control	(2) Furazol-idone	(3) neo-mycin*	(4) Neomycin* + furazolid-one	P (cf 1&2)	P (cf 1&3)	P (cf 3&4
duodenal mucosa	41.51±1.20 (3)	20.34±1.08 (3)	39.52±2.80 (4)	26.76±1.60 (4)	<0.001	>0.1	<0.05
liver	19.52±0.51 (3)	15.32±0.53 (3)	20.85±1.10 (4)	16.81±1.60 (4)	<0.001	>0.1	>0.05
heart	0.58±0.03 (3)	0.31±0.04 (3)	0.49±0.01 (4)	0.30±0.01 (4)	<0.001	>0.05	<0.05
brain	10.12±0.20 (3)	6.11±0.11 (3)	9.48±0.26 (4)	9.10±0.59 (4)	<0.001	>0.1	>0.1

The values in the table are means ± s.e.m. (no. of ducklings).

* neomycin, 20 mg/bird by crop tube, twice daily for 5 days before the Monoamine oxidase estimation.

Table 8. Effect of furazolidone on the activity of aminopyrine demethylase in chicken livers

Exp.	Treatment	Aminopyrine demethylase (H.CHO μ mol ml^{-1}* h^{-1})
1	Control feed	0.37 ± 0.04 (6)
	Furazolidone, o.04% w/w in feed, 10 days	0.37 ± 0.05 (6)
2	Acacia mucilage (crop tube)	0.30 ± 0.03 (5)
	Furazolidone, 200 mg/kg (crop tube)	0.35 ± 0.08 (6)

* per ml of 10,000 g supernatant of liver homogenate (25% w/v).

The values in the table are means ± s.e. means (No. of animals).

The drug had no significant effect on the activity of aminopyrine demethylase.

Table 9. The amounts of 5-hydroxytryptamine (5-HT), noradrenaline and adrenaline in the brains of chicks and ducklings given furazolidone (0.04% w/w) in the feed for 10 days

	Brain amine (μg/g tissue)		
	5-HT	noradrenaline	adrenaline
control chicks	0.55 ± 0.02 (7)	0.20 ± 0.02 (7)	0.10 ± 0.01 (7)
furazolidone-fed chicks	0.67 ± 0.02 (7)**	0.19 ± 0.02 (7)	0.11 ± 0.01 (7)
control ducklings	0.39 ± 0.04 (5)	0.24 ± 0.02 (5)	0.18 ± 0.01 (5)
furazolidone-fed ducklings	0.57 ± 0.06 (5)*	0.18 ± 0.01 (5)	0.17 ± 0.02 (5)

The values in the table are means ± s.e.m. (numbers of birds).

Brain 5-HT was increased in furazolidone-fed birds, *$P < 0.05$, **$P < 0.001$.

Table 10. Effect of furazolidone (0.04% w/w, 10 days) on the vasopressor action of tyramine given intraduodenally in anaesthetised preparations of chickens.

Tyramine HCl (mg/kg)	Vasopressor response (mm Hg) controls	Furazolidone-treated	P furazolidone treatment
5	11.0 ± 2.1 (5)	22.0 ± 3.4 (7)	< 0.05
25	26.3 ± 2.8 (6)	44.8 ± 4.5 (6)	< 0.01

The values in the table are means ± s.e.m. (number of preparations).

Table 11. Effect of methergoline on the feed intake and growth of chickens treated with furazolidone

	(1) Control	(2) furazolidone*	(3) methergoline**	(4) furazolidone + methergoline	P $\bar{c}f$ (1&2)	P $\bar{c}f$ (1&3)	P $\bar{c}f$ (2&4)	P $\bar{c}f$ (3&4
feed intake (g feed/kg bird/10 days)	1060.0±110.1 (3)	801.7±47.5 (3)	1103.4±89.2 (3)	908.7±98.1 (3)	< 0.05	>0.1	>0.1	< 0.0
growth (g/kg bird/ 10 days)	216.6±20.7 (3)	52.3±13.3 (3)	261.9±10.3 (3)	25.4±11.3 (3)	< 0.05	>0.1	>0.1	< 0.0

The values in the table are means ± s.e.m. (number of birds).

* furazolidone (0.04% w/w) was added to the feed for 10 days.

** methergoline was dissolved in 2% w/v ascorbic acid and injected intramuscularly at a dose of 1 mg/kg twice daily.

Table 12. The influence of thiamin on the concentrations of pyruvate and lactate in the blood of control and furazolidone-treated chickens

	(1) control	(2) furazolidone*	(3) thiamin**	(4) furazolidone+ thiamin	P ($\bar{c}$f 1&2)	P ($\bar{c}$f 1&3)	P ($\bar{c}$f 2&4)	P ($\bar{c}$f 3&4)
pyruvate (mM)	0.11±0.00(5)	0.16±0.01(5)	0.12±0.00(5)	0.14±0.01(6)	<0.002	>0.1	>0.1	<0.01
lactate (mM)	3.48±0.14(5)	4.17±0.19(5)	3.33±0.11(5)	3.70±0.15(6)	<0.02	>0.1	>0.05	>0.05

The values in the table are means ± s.e.m. (number of chickens)

* furazolidone (0.04% w/w) in the feed for 10 days.

** thiamin HCl (84 µg/Kg) was injected intramuscularly twice daily.

Table 13. Transketolase (TK) activity and the thiamin pyrophosphate (TPP) effect in haemolysates from control and furazolidone-treated chickens, and the influence of thiamin thereon

	(1) control	(2) furazolidone**	(3) thiamin***	(4) furazolidone+ thiamin	P ($\bar{c}$f 1&2)	P ($\bar{c}$f 1&3)	P ($\bar{c}$f 3&4)	P ($\bar{c}$f 2&4)
TK activity (u/l)	57.7±1.1(5)	52.1±2.8(5)	59.2±1.8(5)	54.1±1.8(6)	>0.1	>0.1	>0.1	>0.1
TPP effect* (%)	7.3±1.4(5)	15.0±1.4(5)	5.4±0.6(5)	10.6±0.7(6)	<0.01	>0.1	<0.001	<0.05

The values in the table are means ± s.e.m. (number of chickens)

* the TPP effect is expressed as the % increase in TK activity upon the addition of TPP.

** furazolidone (0.04% w/w) in the feed for 10 days.

*** thiamin HCl (84 µg/Kg) was injected intramuscularly twice daily.

Table 14. The effect of thiamin on the anorexia in chickens treated with furazolidone

	initial age of birds (weeks)	(1) control	(2) furazolidone*	(3) thiamin**	(4) furazolidone+ thiamin	P ($\bar{c}$f 1&3)	P ($\bar{c}$f 2&4)
feed intake (g/Kg bird/ 10 days)	8	1042.1	875.4	1074.1	864.5	-	-
	11	882.9	733.3	858.3	808.7	-	-
growth (g/Kg Bird/ 10 days)	8	214.7±21.7(3)	85.0±11.5(3)	266.8±13.7(3)	111.3±11.4(3)	>0.1	>0.1
	11	160.9±1.7(3)	50.8±6.1(3)	152.4±4.3(3)	56.0±9.7(3)	>0.1	>0.1

The values are means ± s.e.m. (number of chickens)

* furazolidone (0.04% w/w) in the feed for 10 days

** thiamin HCl (84 µg/Kg) was injected intramuscularly twice daily

Table 15. The effect of thiamin on the monoamine oxidase (MAO) activity in chickens treated with furazolidone

	MAO activity (4-hydroxyquinoline, μ mol/g tissue/h)					
	(1) control	(2) furazolodine*	(3) thiamin**	(4) furazolidone+ thiamin	P ($\bar{c}$f 1&2)	P ($\bar{c}$f 2&4)
caecal mucosa	39.55±0.45(3)	16.35±0.35(3)	38.14±3.94(3)	17.87±0.85(3)	<0.001	>0.1
heart	1.47±0.05(3)	0.89±0.06(3)	1.33±0.06(3)	0.90±0.06(3)	<0.001	>0.1
brain	9.37±0.32(3)	5.72±0.01(3)	10.46±0.96(3)	5.49±0.78(3)	<0.001	>0.1

The values in the table are means ± s.e.m. (number of chickens)

* furazolidone (0.04% w/w) in the feed for 10 days

** thiamin HCl (84 μg/Kg) was injected intramuscularly twice daily

Table 16. Effect of furazolidone (0.04% w/w, 10 days) on catecholamines in the adrenal glands of cockerels (aged 9 weeks at slaughter).

	control	furazolidone	P (treatment)
noradrenaline (µg/adrenal glands)	260 ± 41 (4)	347 ± 55 (6)	>0.1
noradrenaline (µg/g tissue)	4790 ± 1700 (4)	4810 ± 900 (6)	>0.1
adrenaline (µg/adrenal glands)	240 ± 34 (4)	366 ± 60 (6)	>0.1
adrenaline (µg/g tissue)	2670 ± 400 (4)	2440 ± 500 (6)	>0.1

The values in the table are means ± s.e.m. (number of chickens)

Table 17. Effect of furazolidone (0.04% w/w, 10 days) on concentrations of cholestrol and ascorbic acid in the adrenal glands, and cholestrol and glucose in plasma in cockerels (9 weeks old at slaughter).

	control	furazolidone	P (treatment)
adrenal cholestrol (mg/g tissue)	35 ± 1 (5)	32 ± 2 (5)	>0.05
adrenal ascorbic acid (µg/100 mg tissue)	271 ± 22 (5)	267 ± 13 (5)	>0.1
plasma cholestrol (mg/100 ml)	154 ± 4 (5)	154 ± 6 (5)	>0.1
plasma glucose (mM)	11.0 ± 0.2 (6)	11.4 ± 0.4 (6)	>0.1

The values in the table are means ± s.e.m. (number of chickens)

Table 18. The effect of furazolidone (0.04% w/w, 10 days) on organ weights in cockerels (9 weeks)

	Organ Weights			
	g		% body weight	
	control	furazolidone	control	furazolidone
adrenal glands	0.0846 ± 0.0048(5)	0.1002 ± 0.0166(5)	0.0115 ± 0.0004(5)	0.0168 ± 0.0019(5)*
heart	3.1400 ± 0.1200(5)	2.5700 ± 0.2400(6)	0.4295 ± 0.0133(5)	0.4443 ± 0.0310(6)
pancreas	1.9900 ± 0.0900(5)	1.8200 ± 0.1600(5)	0.2716 ± 0.0092(5)	0.3141 ± 0.0217(6)
thyroid glands	0.0816 ± 0.0034(5)	0.0775 ± 0.0071(6)	0.0111 ± 0.0002(5)	0.0133 ± 0.0006(6)**

The values in the table are means ± s.e.m. (number of chickens).

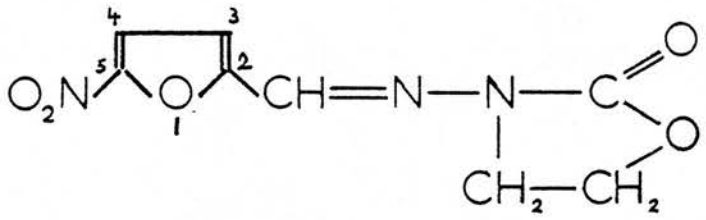
The asterisks mark a significant increase in organ weights in the furazolidone-treated chickens (*, $P < 0.05$; **, $P < 0.01$).

Table 19. The effect of furazolidone on the arterial blood pressure, blood haemoglobin (Hb), packed cell volume (PCV) and plasma aspartate transaminase activity in conscious chickens

	control	furazolidone*	P (treatment)
arterial blood pressure (mm Hg)	127.0 ± 2.0(7)	120.0 ± 3.0(7)	>0.1
Hb content (g%)	8.4 ± 0.5(4)	8.6 ± 0.3(4)	>0.1
PCV (%)	26.8 ± 1.0(4)	27.1 ± 0.9(4)	>0.1
aspartate transaminase activity (iu/l)	56.2 ± 5.2(5)	59.1 ± 5.2(8)	>0.1

The values in the table are means ± s.e.m. (number of chickens).

* furazolidone (0.04% w/w) in the feed for 10 days.



Furazolidone

Figure 1. Chemical structure of furazolidone ((N-5-nitro-2-furfurylidene)3-amino-2-oxazolidine)

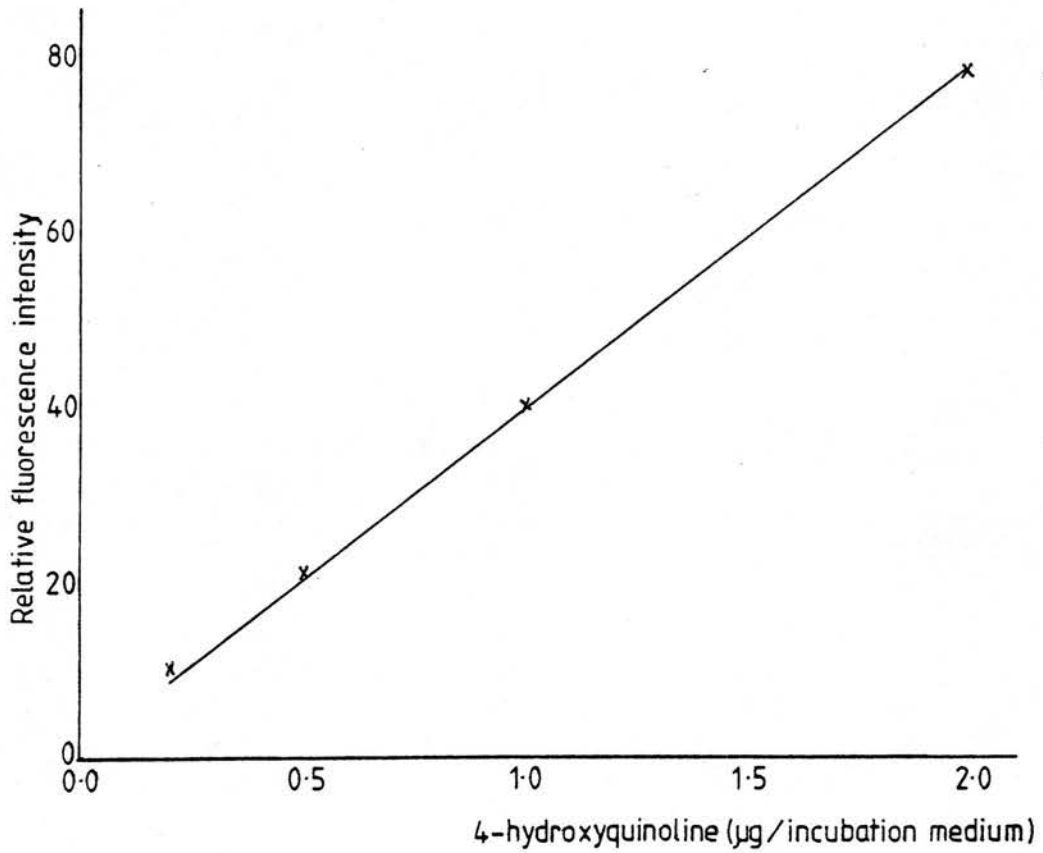


Figure 2. A calibration curve for the determination of 4-hydroxyquinoline produced by tissue homogenates from the oxidative deamination of kynuramine. Ordinate : relative fluorescence intensity; abscissa : concentrations of 4-hydroxyquinoline (µg/ incubation medium).

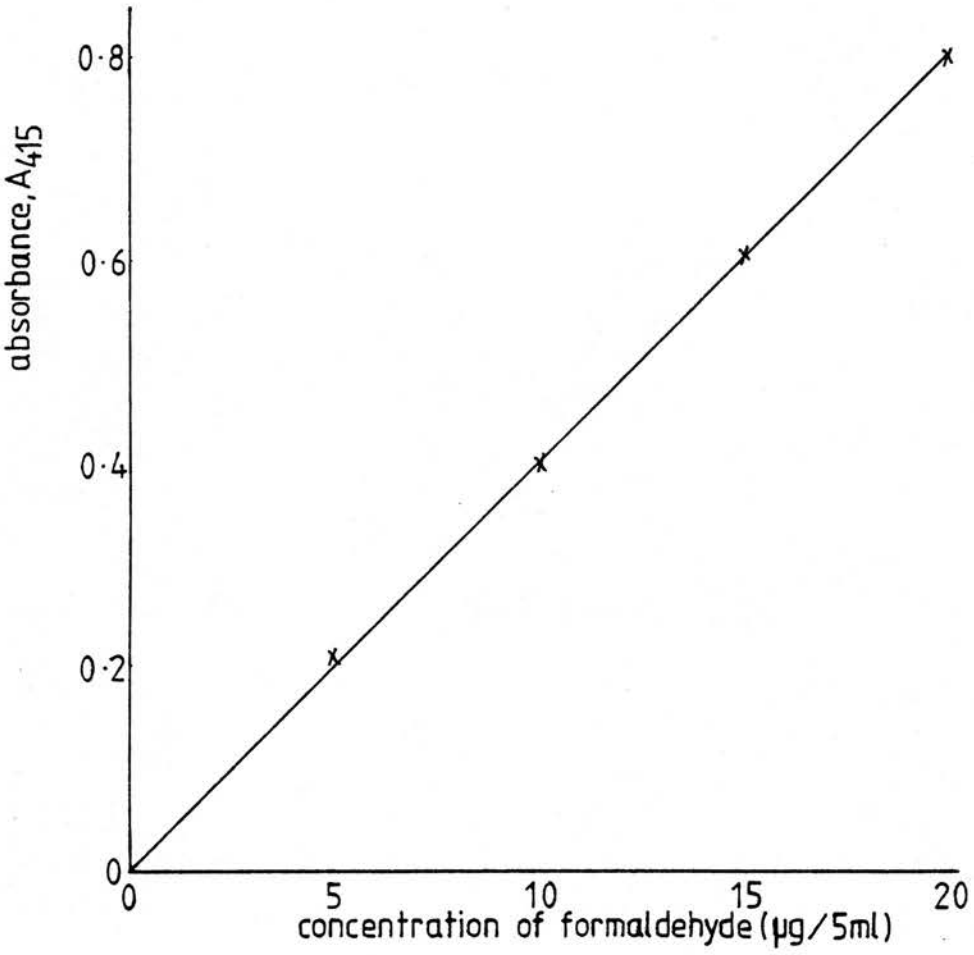


Figure 3. A calibration curve for the determination of formaldehyde produced by chicken liver supernatant fraction incubated with aminopyrine. Ordinate : absorbence (A) measured at a wavelength of 415 nm; abscissa : concentrations of formaldehyde (µg/5 ml) reacted with 'Nash Reagent'.

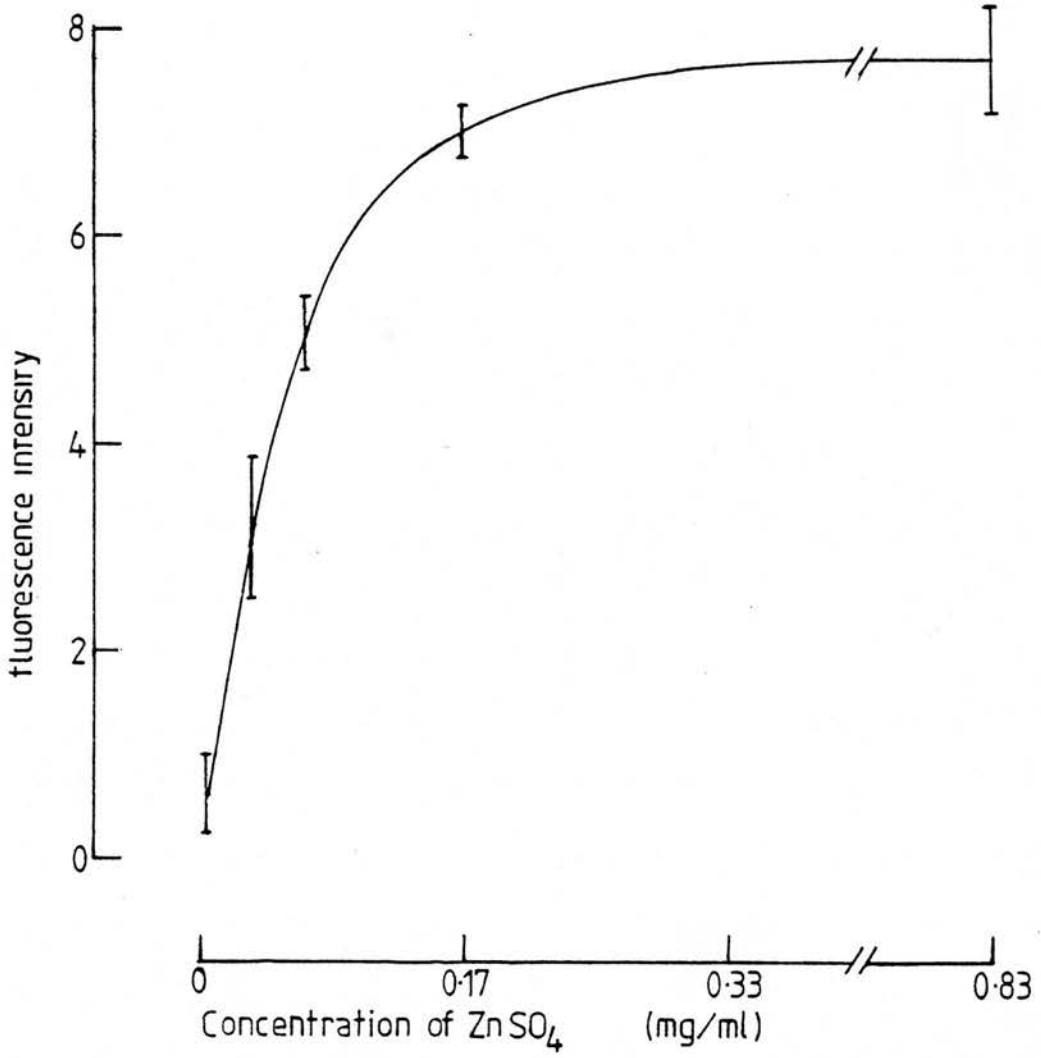


Figure 4. The influence of $ZnSO_4$ on the rate of oxidation of adrenaline (1 µg/ml). Ordinate : fluorescence intensity arising from the oxidation of the amine with potassium ferricyanide for 3 min at pH 3.5; abscissa : final concentration of $ZnSO_4$ in the oxidation mixture (mg/ml). The plotted points are mean values obtained from 4 observations, the s.e.m. being depicted by the vertical bars. The rate of oxidation of adrenaline increased with the increase in concentration of $ZnSO_4$ up to 0.17 mg/ml, thereafter it plateaued.

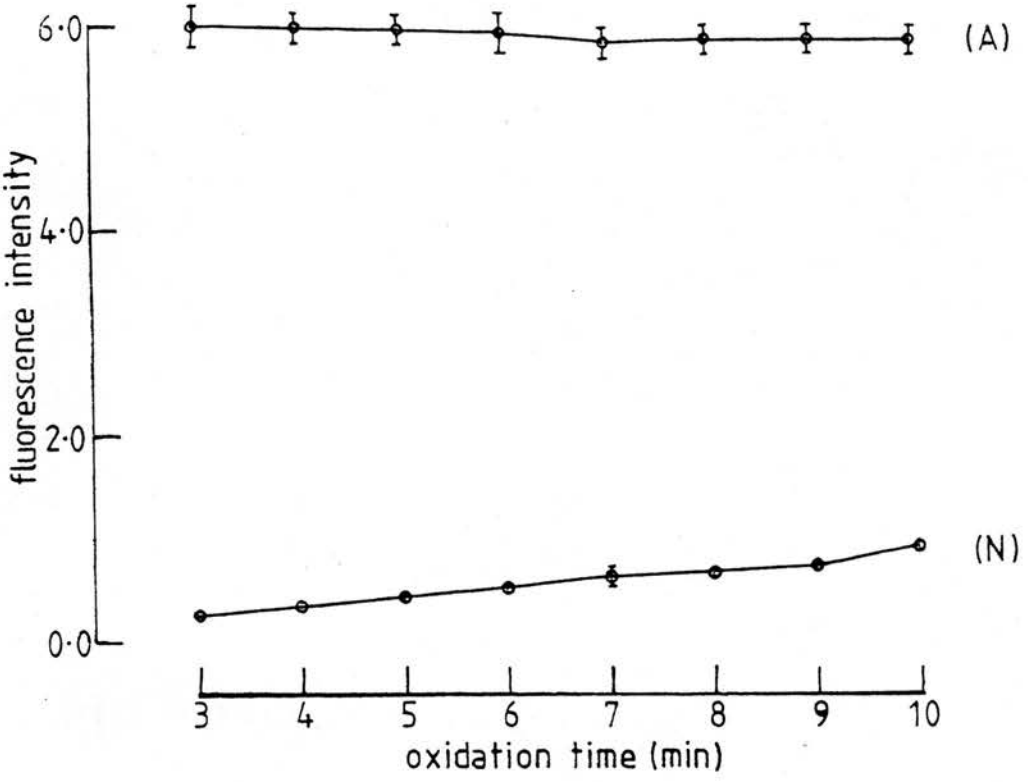


Figure 5. The fluorescence intensity of adrenaline and noradrenaline reacted in the trihydroxyindole reaction in the presence of $ZnSO_4$ (0.17 mg/ml) at pH 3.5. Ordinate : fluorescence intensity; abscissa : time of oxidation with potassium ferricyanide (min). The plotted points are mean values (n = 6) for adrenaline (1 µg/ml) (A) and noradrenaline (1 µg/ml) (N). The vertical bars depict the s.e.m. Maximum fluorescence of adrenaline occurred in 3 min, whereas the fluorescence of noradrenaline increased slowly with time.

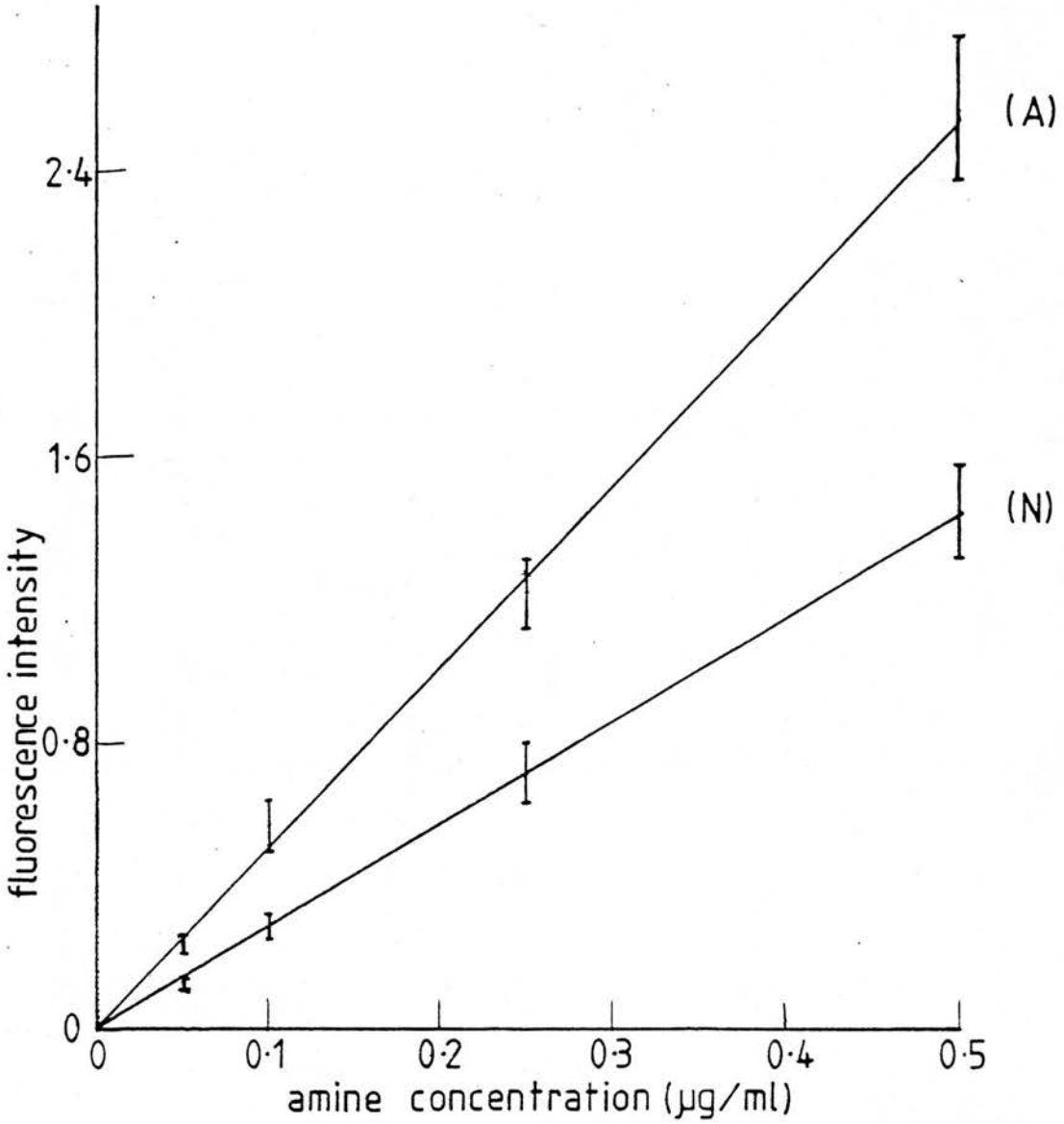


Figure 6. Fluorescence intensity of different concentrations of adrenaline (A) and noradrenaline (N) solutions, oxidized with potassium ferricyanide for 3 min at pH 6.5. Ordinate : fluorescence insensitivity; abscissa : concentrations of the amine (µg base/ml). The plotted points are mean values of 6 observations, the standard errors of the mean being depicted by vertical bars. The regression line of fluorescence upon amine concentrations was calculated by the method of least square.

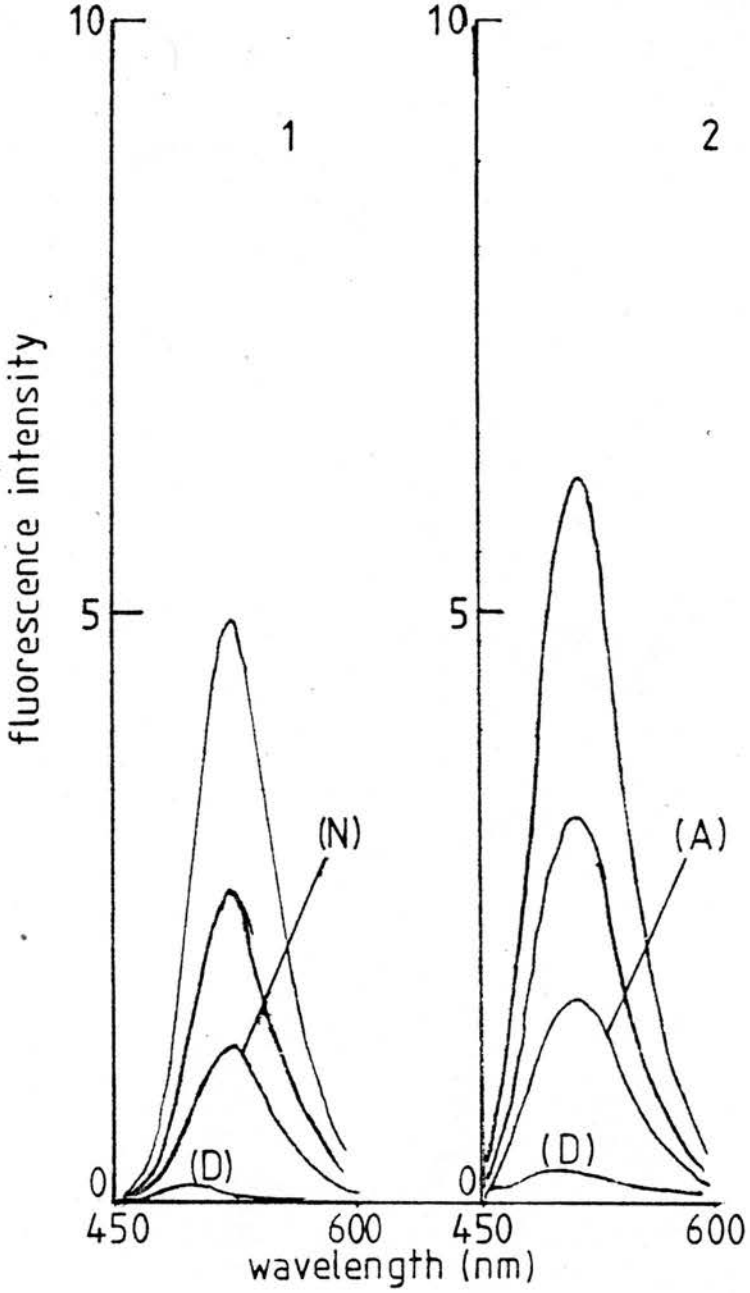


Figure 7. The fluorescence spectra of noradrenaline (N), adrenaline (A) and dopamine (D) reacted by the trihydroxyindole (THI) method. Ordinate : fluorescence intensity; abscissa : emission wavelength (450 - 600 nm). From above down in (1), the fluorescence spectra from noradrenaline 1.00, 0.50 and 0.25 µg/ml and dopamine 100 µg/ml, reacted by THI method at pH 6.5. From above down in (2), the fluorescence spectra from adrenaline 1.00, 0.50 and 0.25 µg/ml and dopamine 100 µg/ml reacted by the THI method at pH 3.5. Dopamine produced fluorescence spectra similar to that of the reagent blank.

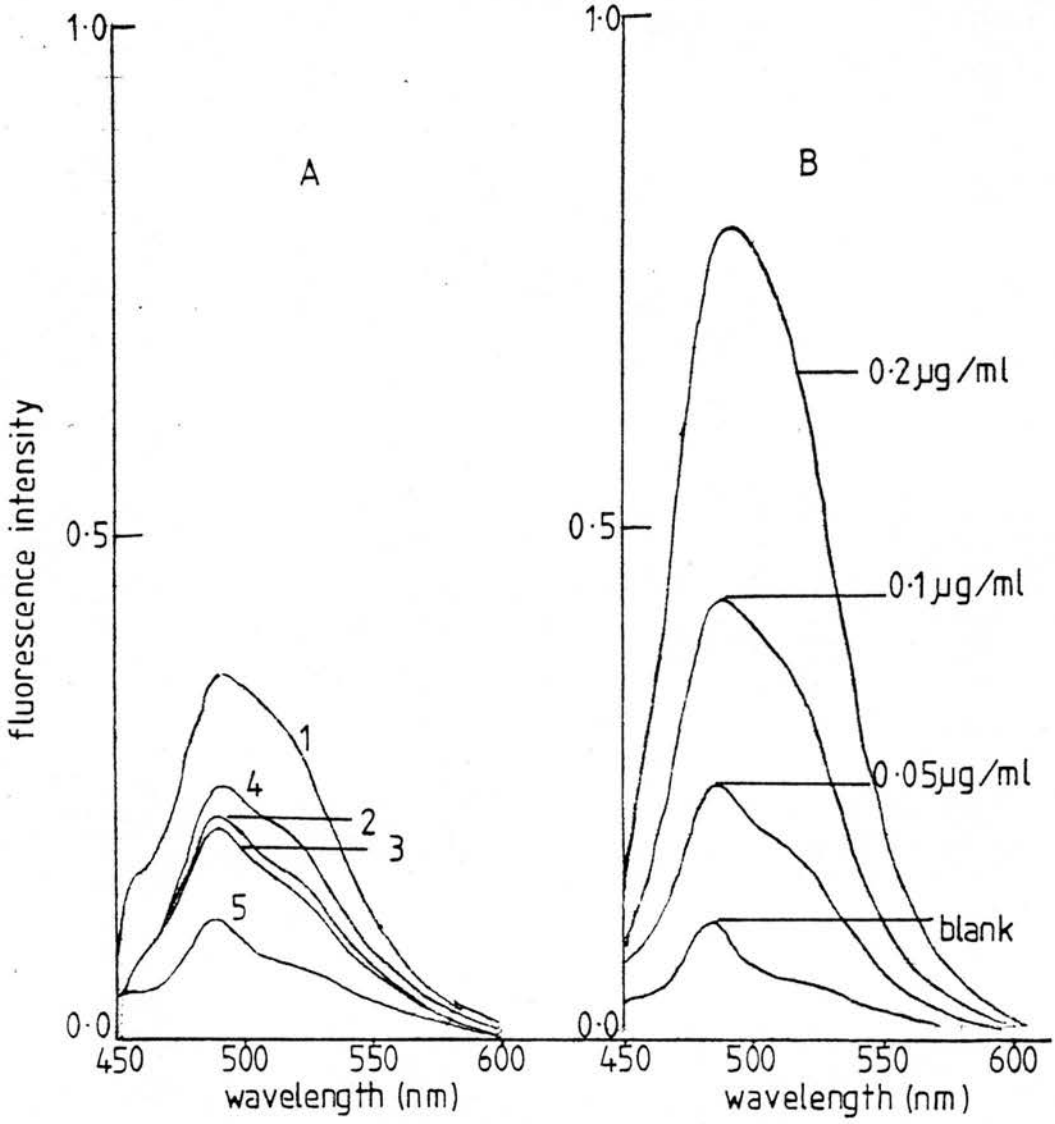


Figure 8. The fluorescence of noradrenaline in extracts of chicken brain and from authentic solutions of the amine reacted by the 'trihydroxyindole' method. Ordinate : fluorescence insensitivity; abscissa : emission wavelength (450 - 600 nm). The spectra labelled 1 and 2 in (A) were obtained from brain extracts oxidized at pH 6.5 and 3.5 respectively. Spectra (3), (4) and (5) are those of noradrenaline (0.05 µg/ml), noradrenaline (0.1 µg/ml) and reagent blank, respectively. The fluorescence spectra in (B) were obtained from noradrenaline solutions, passed through the CG50 columns. The peaks of the spectra of noradrenaline shifted from 485-493 nm as the amounts of the amine increases from 0.05 - 0.2 µg/ml.

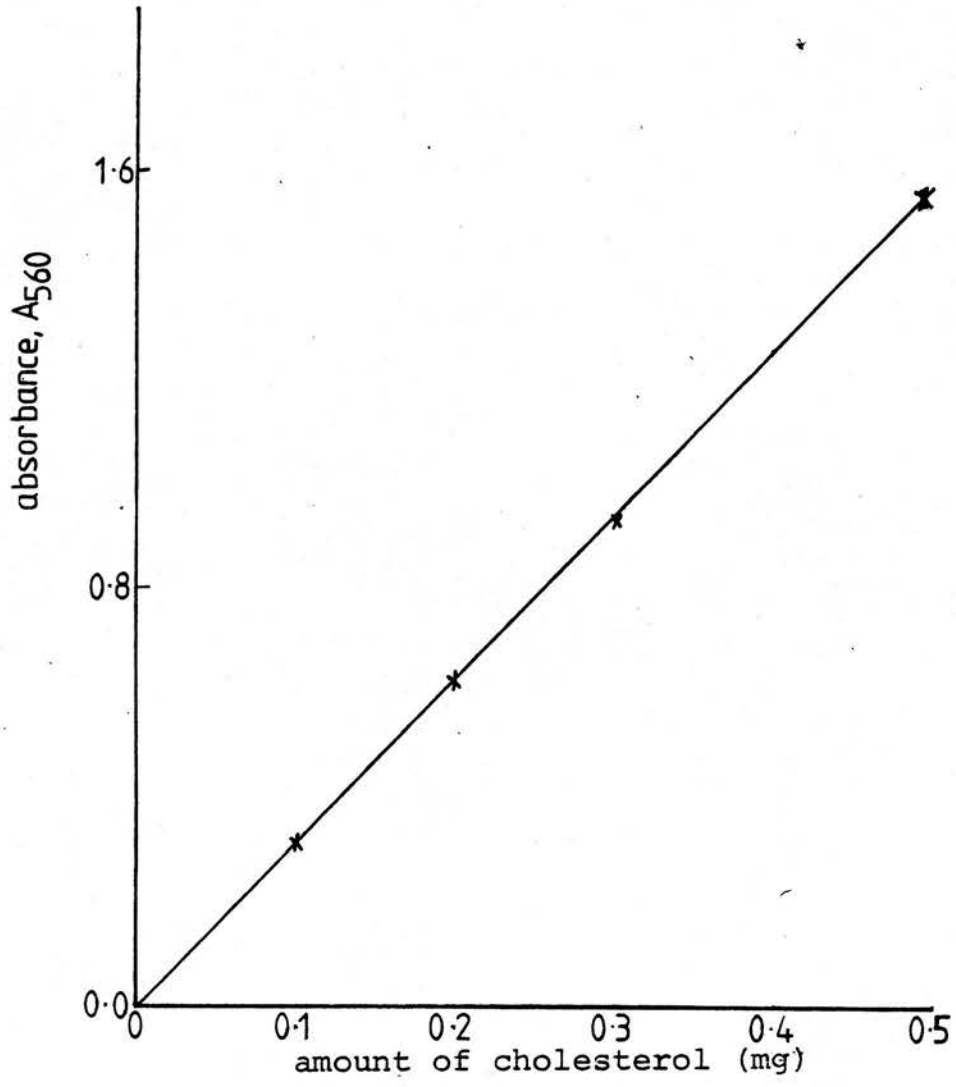


Figure 9. A calibration curve for the determination of cholesterol concentration in plasma. Ordinate : absorbance (A) measured at a wavelength of 560 abscissa : amount of cholesterol (mg) in the mixture.

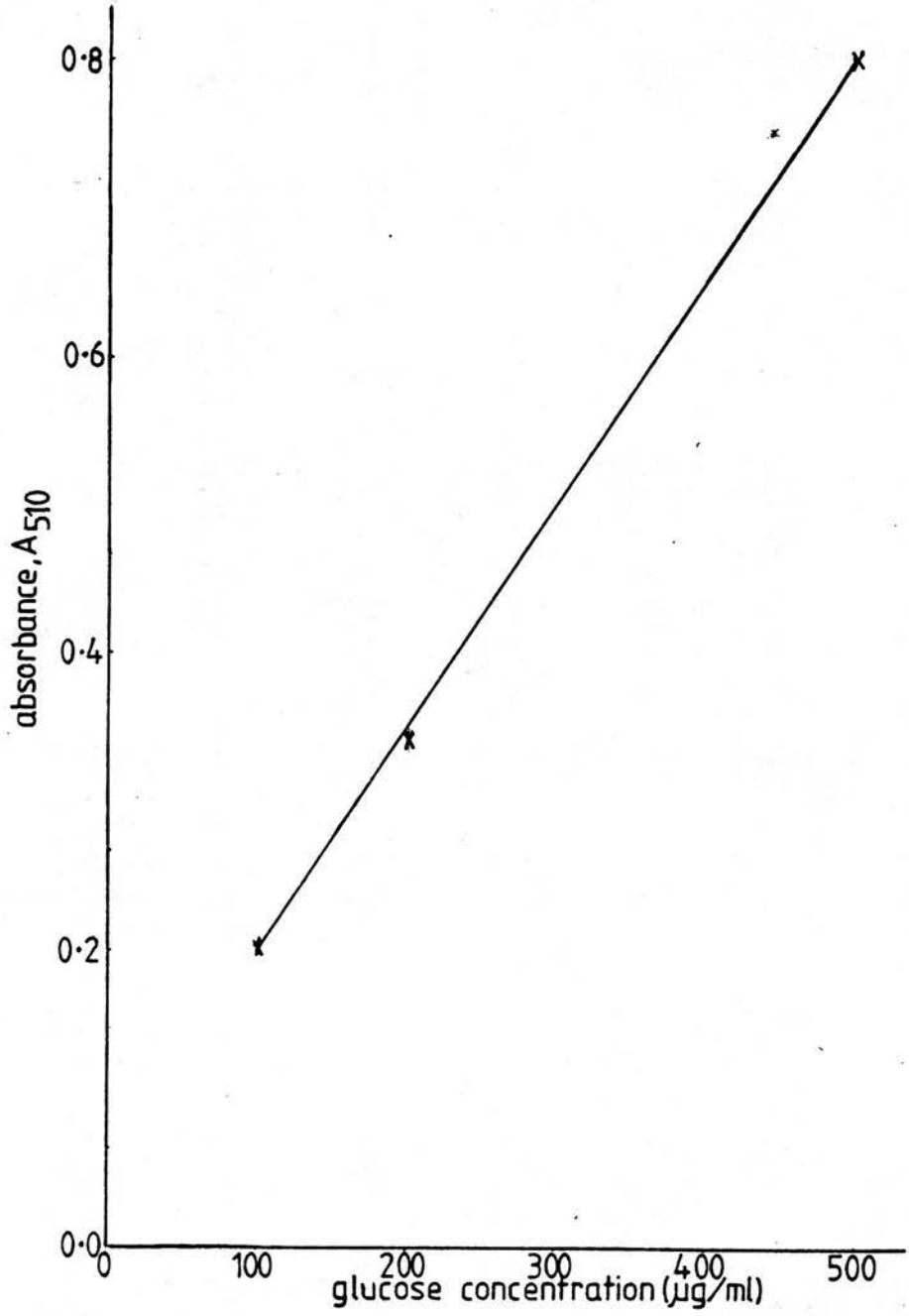


Figure 10. A calibration curve for the determination of glucose concentration in chicken plasma. Ordinate : absorbance (A) measured at 510 nm; abscissa : concentration of glucose solutions (µg/ml).

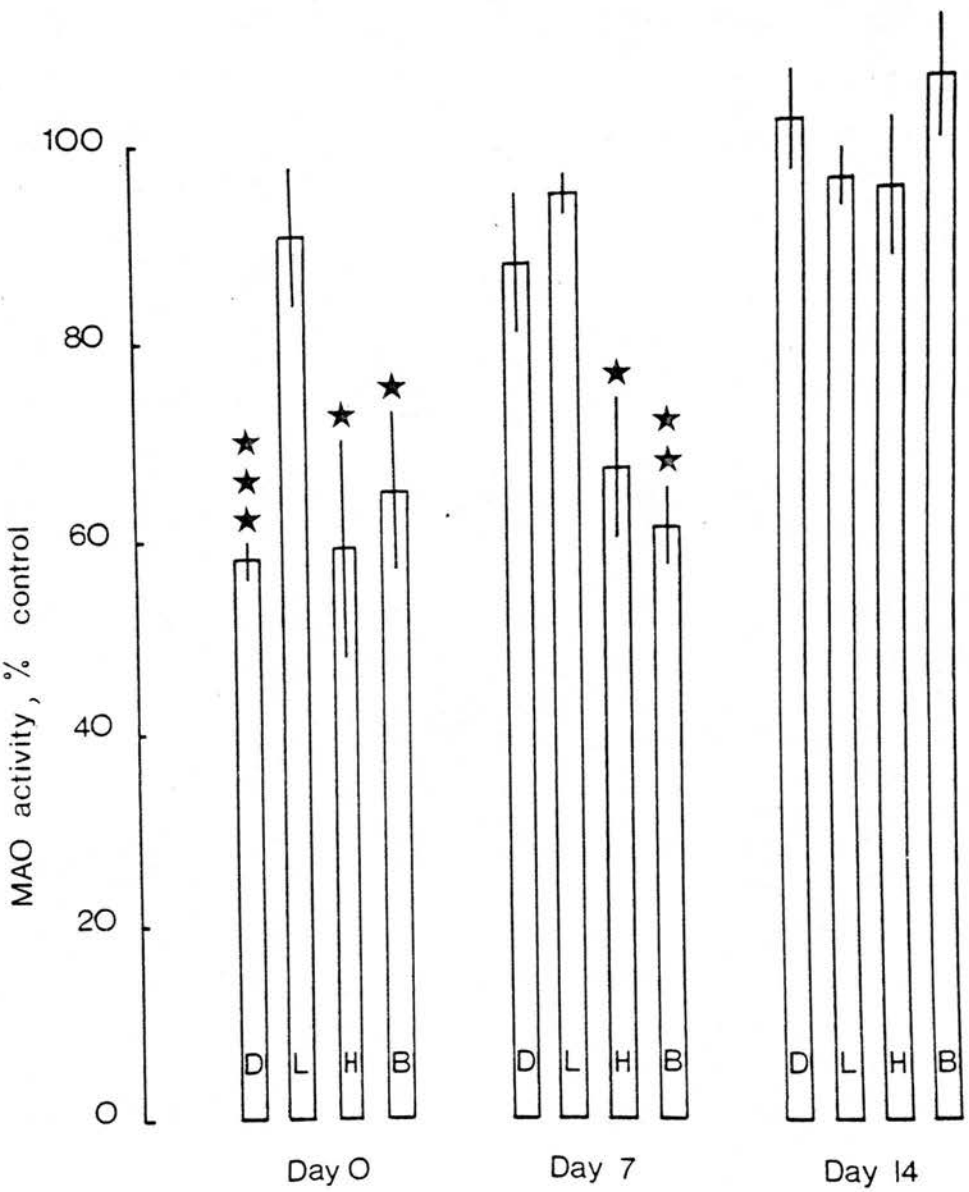


Figure 11. Monoamine oxidase (MAO) activity in homogenates of chicken organs after discontinuing furazolidone in the feed (0.04% w/w, 10 days). Ordinate : MAO activity as a percentage of the control value. The columns represent mean values for duodenal mucosa (D), liver (L), heart (H) and brain (B) at 0, 7 and 14 days after discontinuing furazolidone medication. The vertical bar at the head of each column depicts the standard error of the mean (3 birds). The asterisks mark significant inhibition of MAO, at the 2% (*), 0.2% (**) and 0.1% (***) levels.

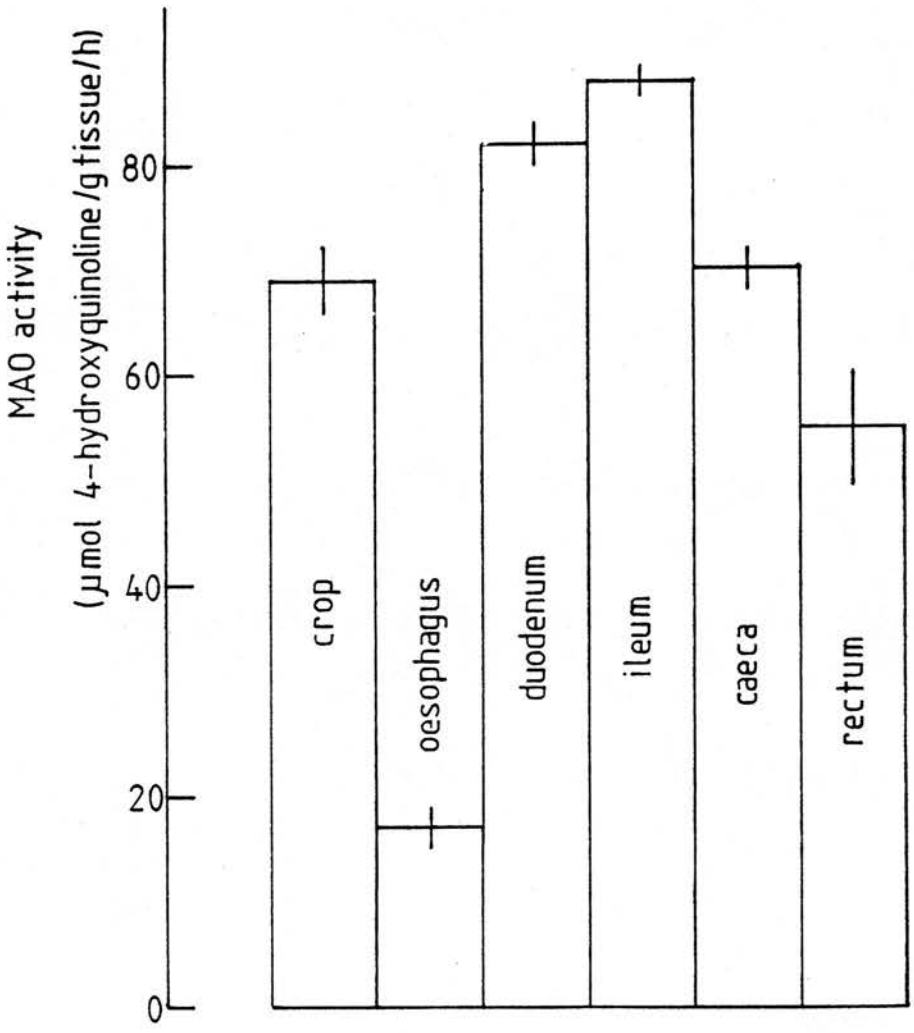


Figure 12. The activity of monoamine oxidase (MAO) in the mucosa of different parts of the chicken alimentary tract. Each column and vertical bar represents a mean value and the s.e.m., respectively, from 3 birds.

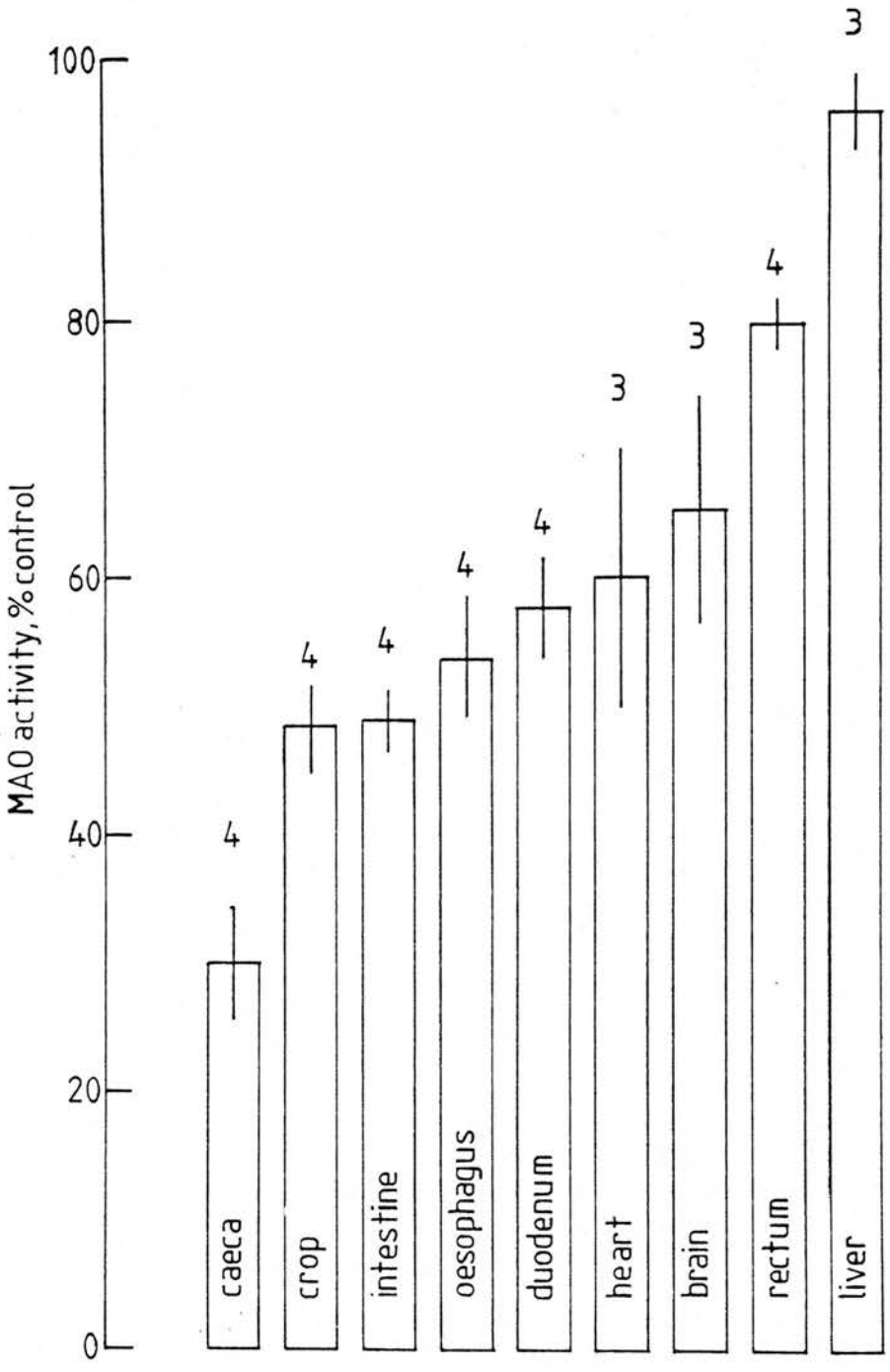


Figure 13. Effect of furazolidone (0.04% w/w in the feed for 10 days) on the activity of monoamine oxidase (MAO) in chicken tissues. Ordinate : MAO activity expressed as % control. Each column and vertical bar depicts a mean and the number of birds used. The residual enzyme activity in the caeca was less than that in other tissues ($P < 0.02 - < 0.001$), and that in the liver was not different from the control ($P > 0.1$).

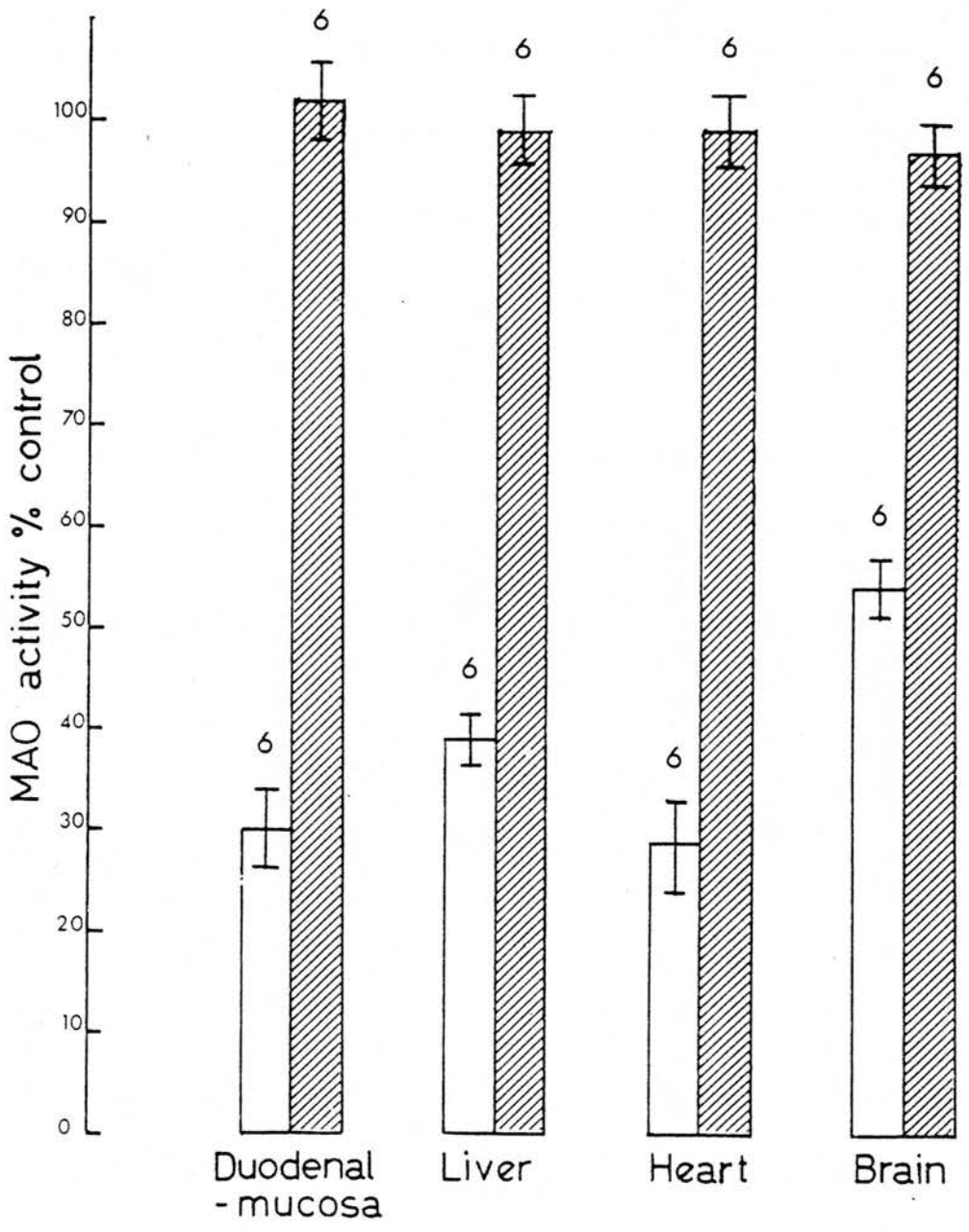


Figure 14. The activity of monoamine oxidase (MAO) in homogenates of chicken organs 24 h after giving the birds a single oral (open columns) or intramuscular (cross-hatched columns) dose of furazolidone (200 mg/Kg). Ordinate : MAO activity expressed as % control. Each column represents a mean value obtained from 6 birds, the s.e.m. being depicted by vertical bars. Intramuscular furazolidone did not affect the activity of MAO significantly in any of the organs studied ($P > 0.1$).

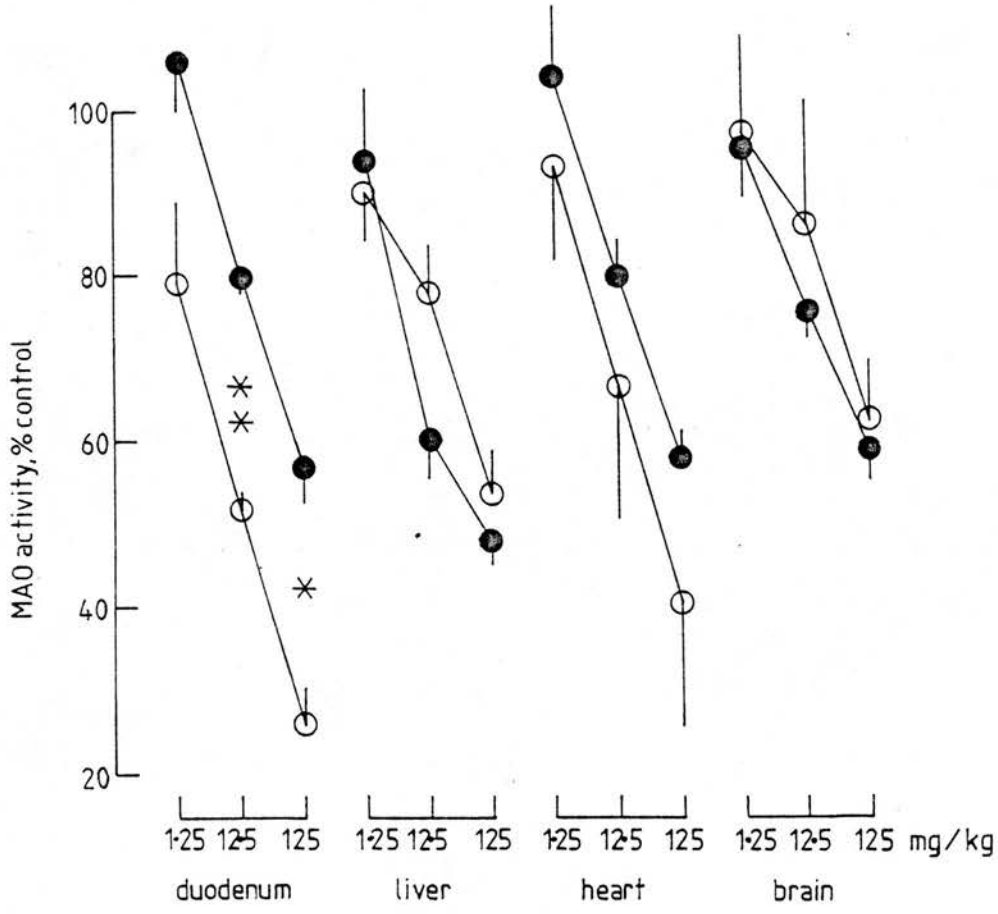


Figure 15. Inhibition of monoamine oxidase (MAO) activity in chicks (closed circles) and ducklings (open circles) 24 h after the administration of furazolidone by crop tube. Ordinate : MAO activity expressed as % control. Abscissa : doses of furazolidone (mg/Kg, on a geometric scale) administered to birds aged 2-12 days. The circles represent mean values obtained from 5 chicks or 3 ducklings, the s.e.m. being depicted by vertical bars. The asterisks mark a species difference in the inhibition of MAO activity in the duodenum, which was significant at the 1% (*) and 0.1% (**) levels.

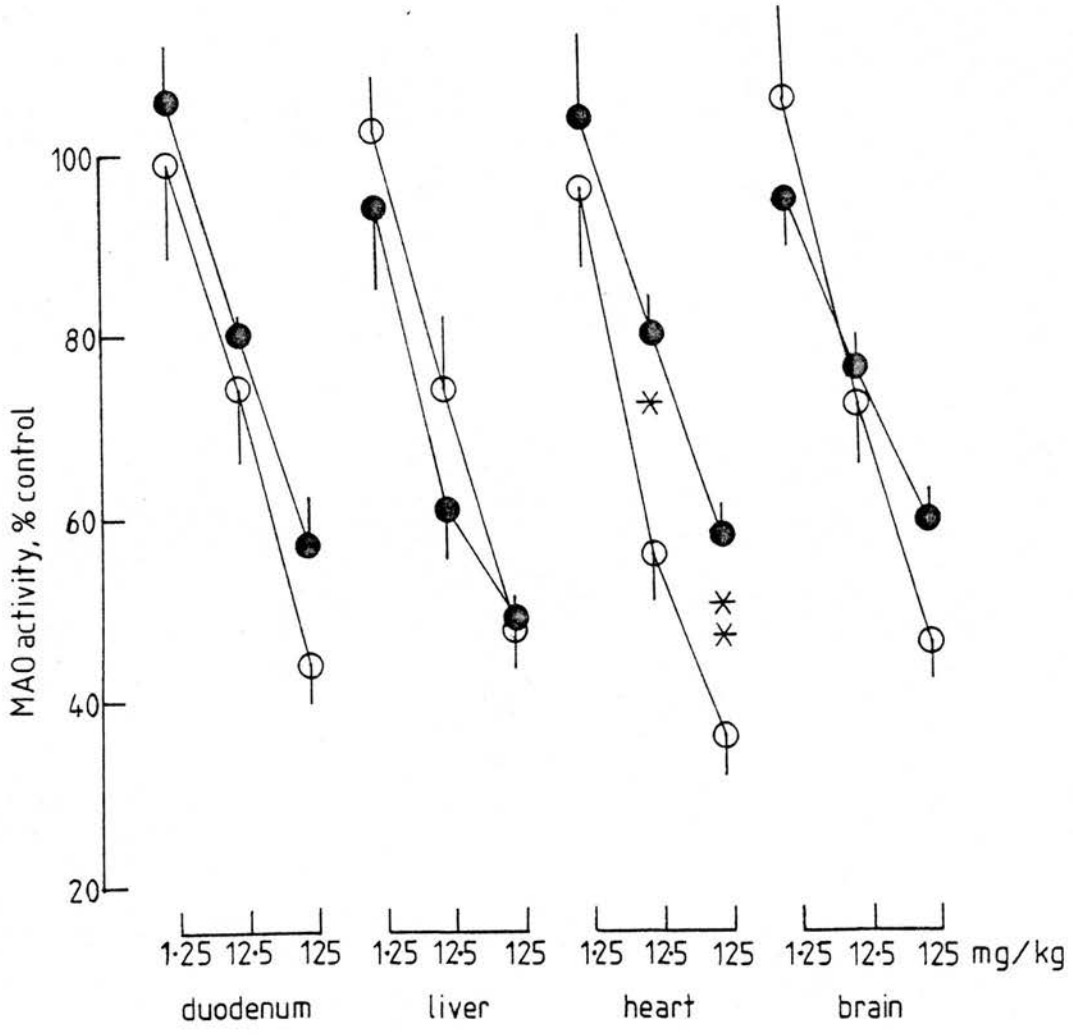


Figure 16. Inhibition of monoamine oxidase (MAO) activity in chicks (closed circles) and turkey poults (closed circles) 24 h after the administration of furazolidone by crop tube.
Ordinate : MAO activity expressed as % control.
Abscissa : doses of furazolidone (mg/Kg, on a geometric scale) administered to birds aged 2-12 days. The circles represent the mean values obtained from 5 chicks or turkey poults, the s.e.m. being depicted by vertical bars. The asterisks mark a species difference in the inhibition of MAO activity in the heart which was significant at the 5% (*) and 1% (**) level.

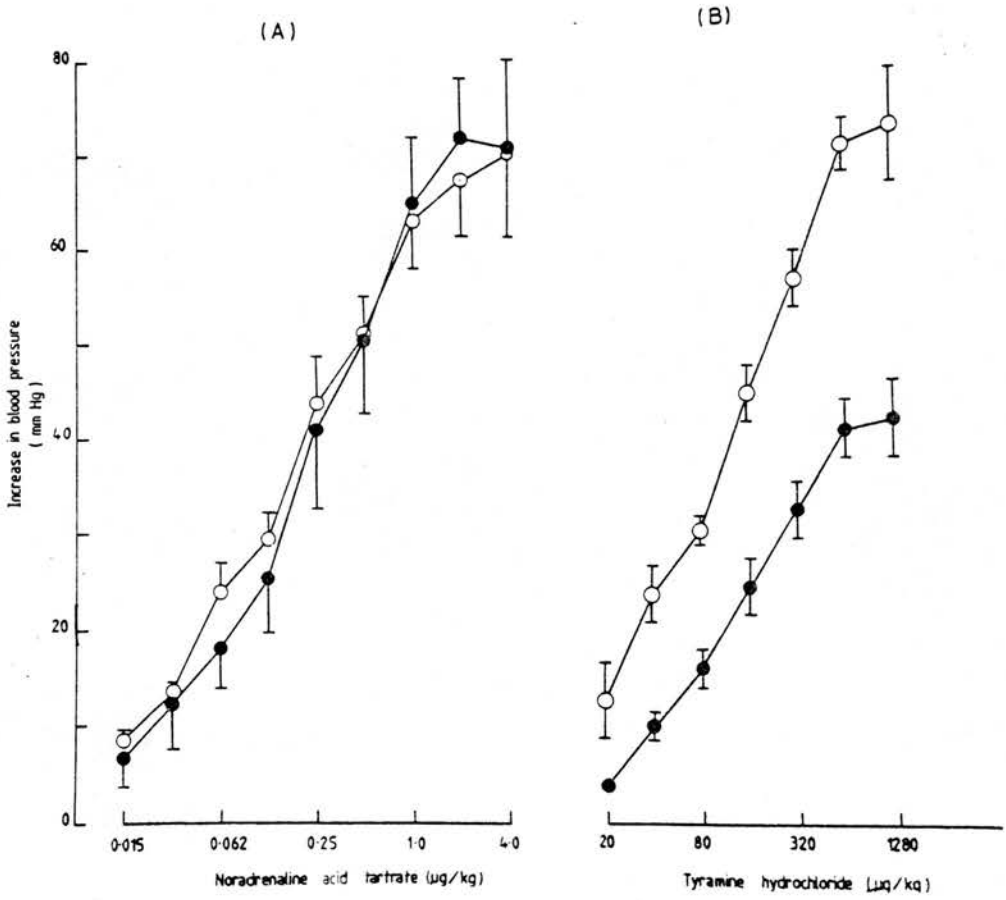


Figure 17. The vasopressor actions of noradrenaline and tyramine injected intravenously in preparation made from control (closed circles) and furazolidone-treated (open circles) chickens anaesthetized with sodium phenobarbitone (180 mg/Kg). Ordinate : increase in arterial blood pressure (mm Hg). Abscissa : doses of noradrenaline acid tartarate and tyramine hydrochloride (µg/Kg, on a geometric scale). Each circle and vertical bar represents a mean value and the s.e.m., respectively, from 4 - 6 pullets. Treatment with furazolidone (0.04% w/w, 10 days) potentiated the action of tyramine ($P < 0.01$) at all but the lowest dose, the response to noradrenaline was unaffected ($P > 0.01$).

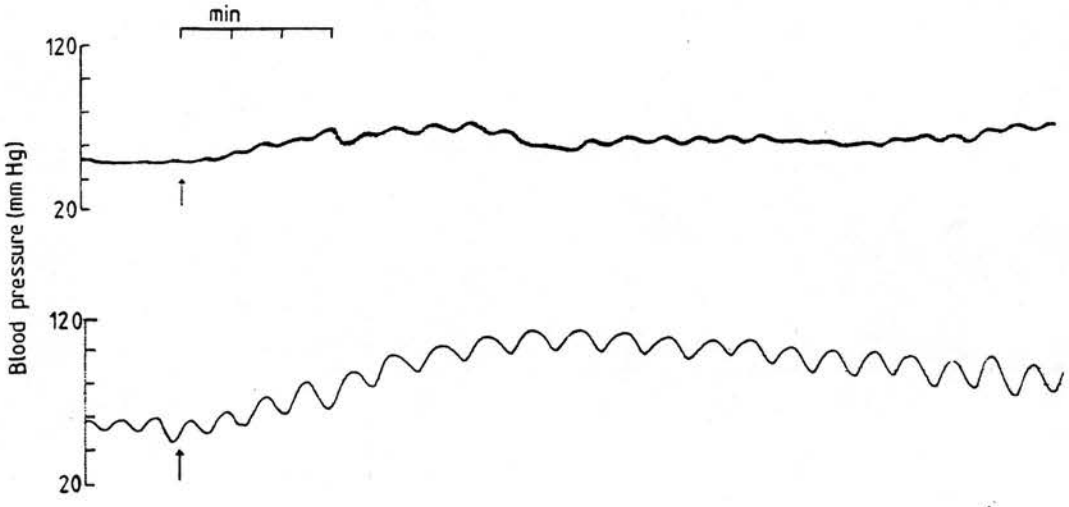


Fig. 18. Arterial blood pressure of anaesthetized preparations of cockerels given a control (upper tracing) or furazolidone-medicated diet (lower tracing). Ordinates: arterial blood pressure (mm Hg). Abscissa: time (min). The arrows mark the administration of tyramine hydrochloride (25 mg/kg) by intraduodenal injection. Treatment with furazolidone (0.04% w/w, 10 days) potentiated the vasopressor action of tyramine.

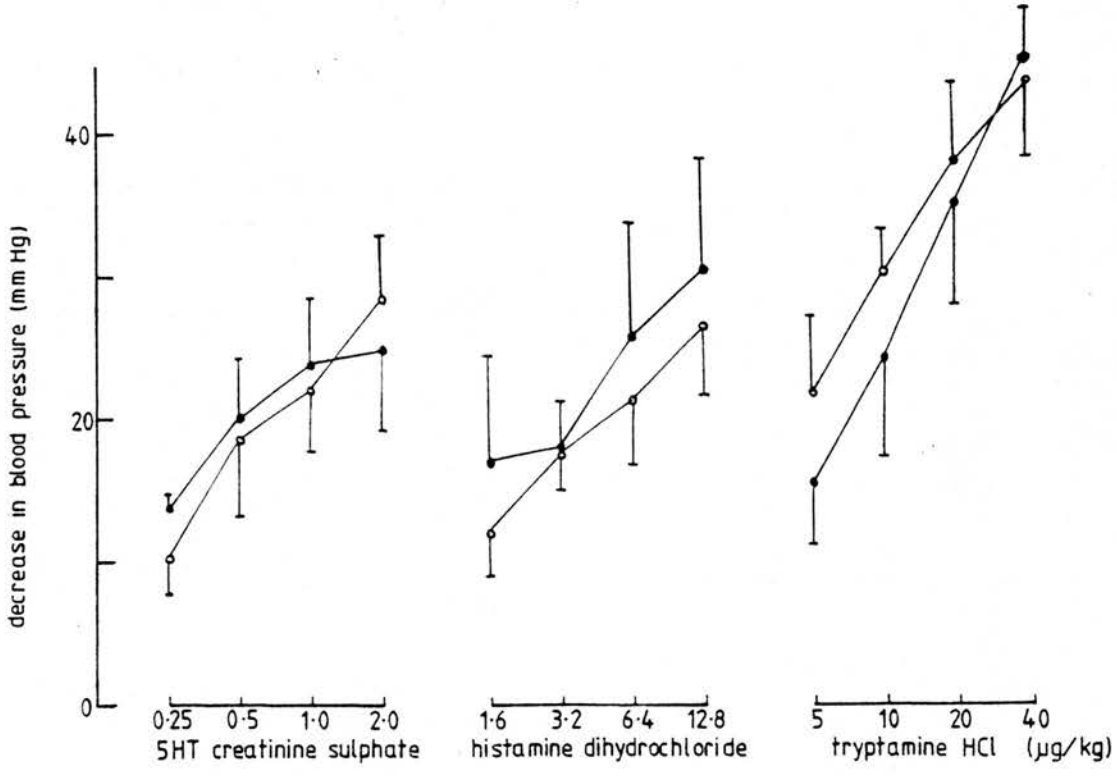


Figure 19. The vaso depressor actions of 5-HT, histamine and tryptamine given intravenously in anaesthetized preparations of control (closed circles) and furazolidone-treated (open circles) chickens. Ordinate : decrease in arterial blood pressure (mm Hg). Abscissa : doses of 5-HT creatinine sulphate, histamine dihydrochloride and tryptamine hydrochloride (µg/Kg, on a geometric scale). The circles and bars represent the mean values and the s.e.m., respectively, from 4-5 birds. Treatment with furazolidone (0.04% w/w, 10 days) did not affect the action of the amines tested ($P > 0.1$).

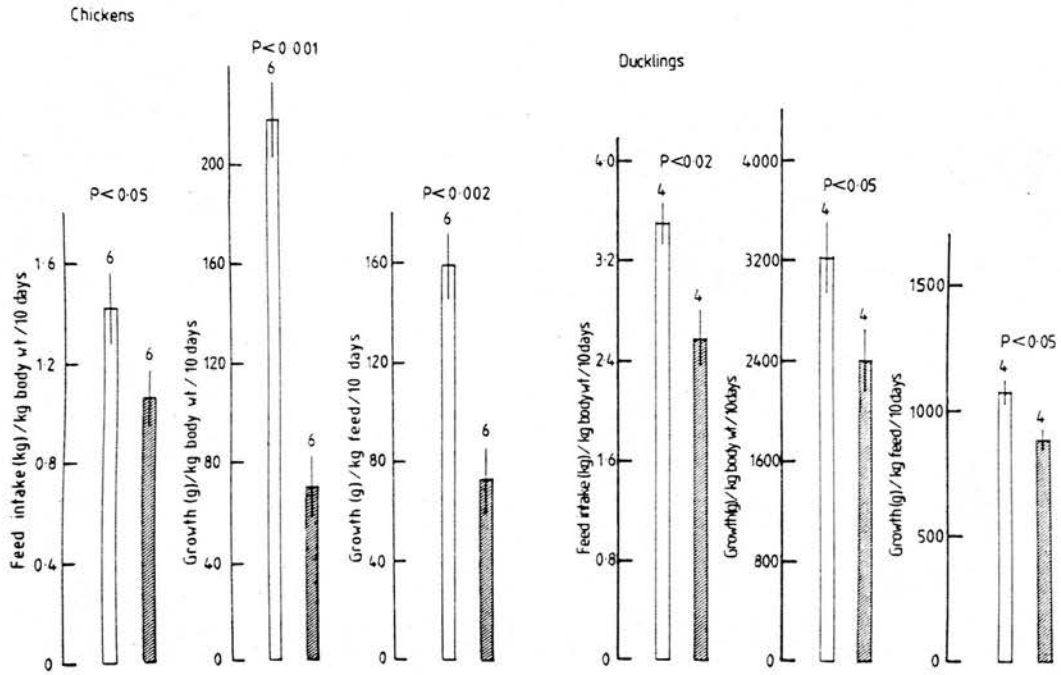


Figure 20. The effect of furazolidone (0.04% w/w in the feed for 10 days) on food consumption and growth in 6 groups of chickens (7½ weeks old at the start of the experiment) and 4 groups of ducklings (4 days at commencement). Ordinate : feed intake (Kg)/Kg body weight/10 days growth (g)/Kg body weight/10 days, and growth (g)(Kg feed/ 10 days). Each column represents a mean value, the s.e.m. being depicted by a vertical bar. The control birds (open columns) consumed more food and grew more than the furazolidone-treated birds (cross-hatched columns).

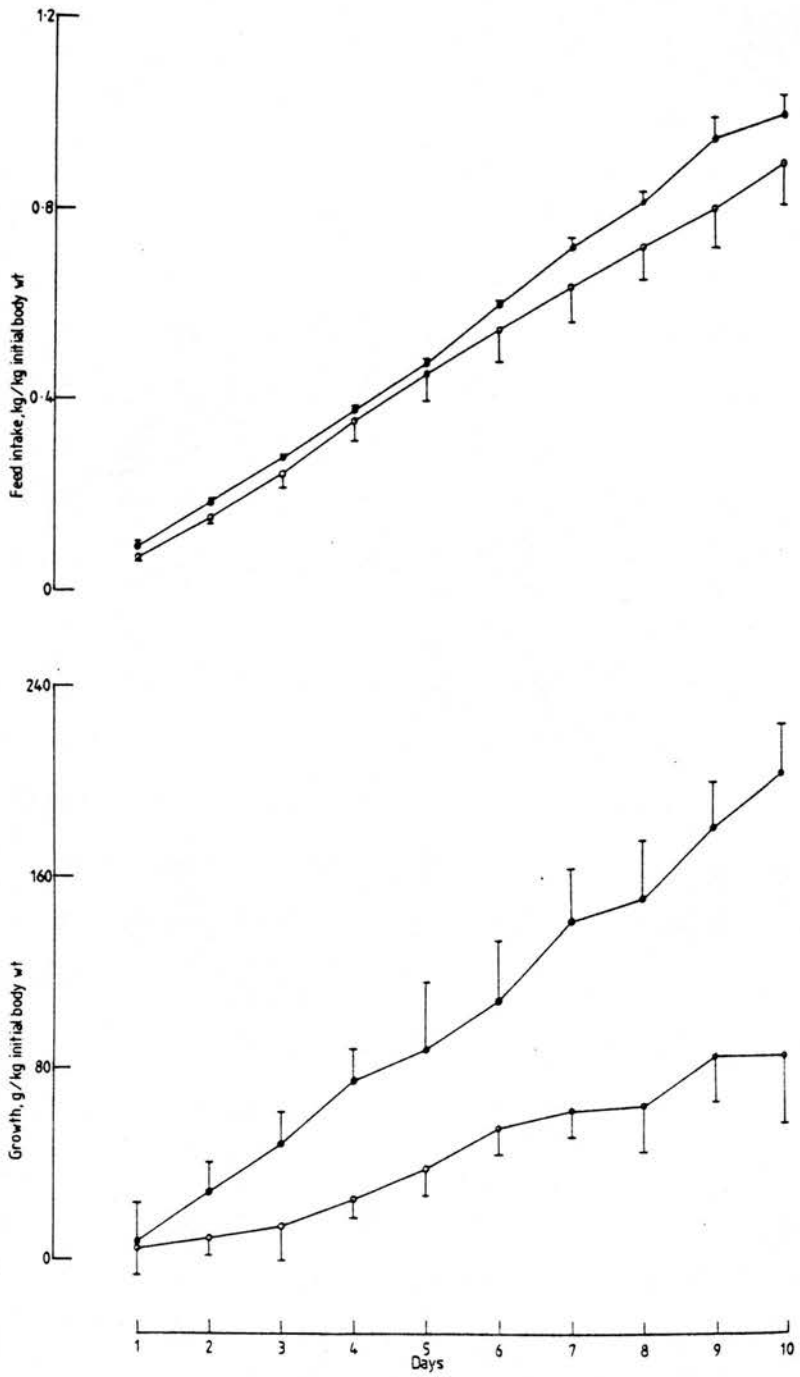


Figure 21. The influence of furazolidone (0.04% w/w, 10 days) on the cumulative feed intake and growth in chickens housed individually (7½ weeks old at the beginning of the experiment, n = 5 or 6). Upper ordinate : feed intake (Kg/Kg initial body weight), lower ordinate : growth (g/Kg initial body weight); abscissa : days of the experiment. In the treated birds (open circles) growth was significantly less than in the controls (closed circles) on days 6 - 10 of the experiment ($P < 0.01$).

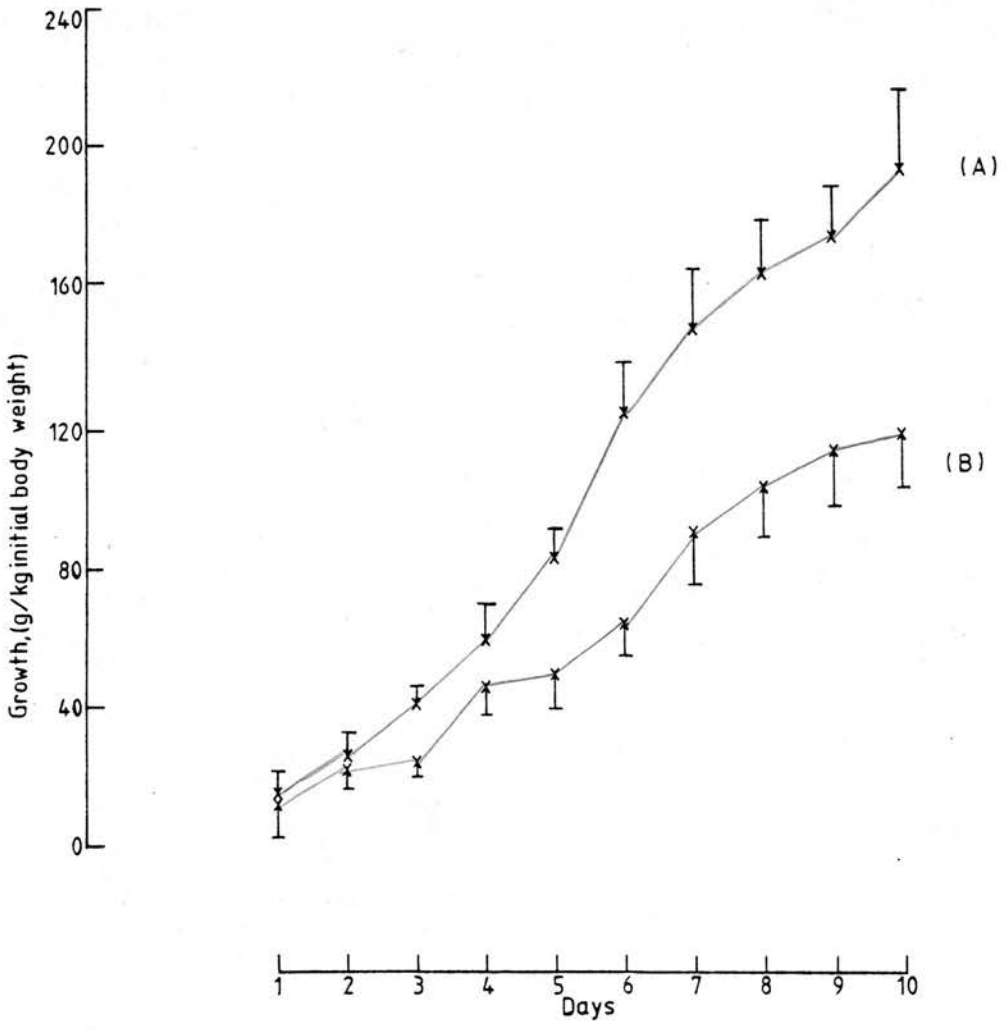


Figure 22. Growth in chickens on restricted diets of unmedicated food. The chickens were pair-fed with birds on (A) unmedicated food, and (B) given furazolidone (0.04%w/w). Ordinate : growth (g/Kg initial body weight); abscissa : days of the experiment. The crosses and vertical bars represent the mean and s.e.m., respectively, for 6 birds. The T-line chickens were 7½ weeks old on day 1 of the experiment. On days 5 to 10 of the experiment, the chickens grew less on diet (B) than on (A) ($P < 0.05 - < 0.02$).

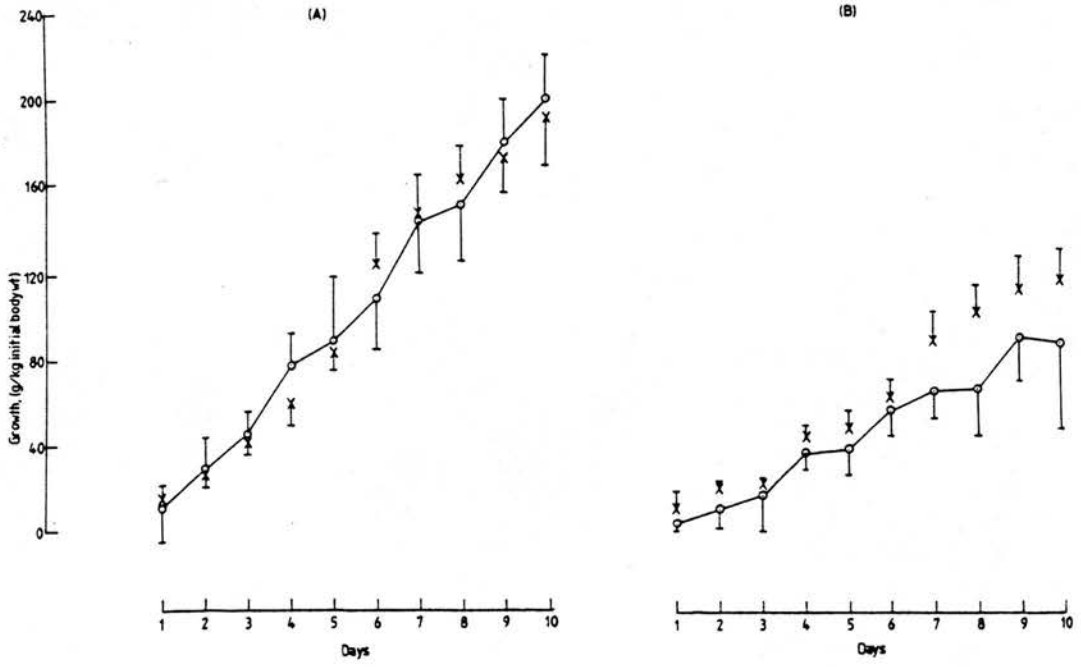


Fig. 23. Growth in chickens fed ad libitum (circles) and pair-fed (crosses) with a restricted diet equivalent to that consumed ad libitum. Ordinate = growth (g/kg initial body weight). Abscissa: days of the experiment. In (A), both groups were on an unmedicated diet. In (B), the chickens fed ad libitum received furazolidone (0.04% w/w) in the diet, and the pair-fed birds on unmedicated feed. The T-line chickens were 7½ weeks old on day 1 of the experiment and they were 6 in each group. There was no significant difference in growth between the ad libitum and the pair-fed birds ($P > 0.1$).

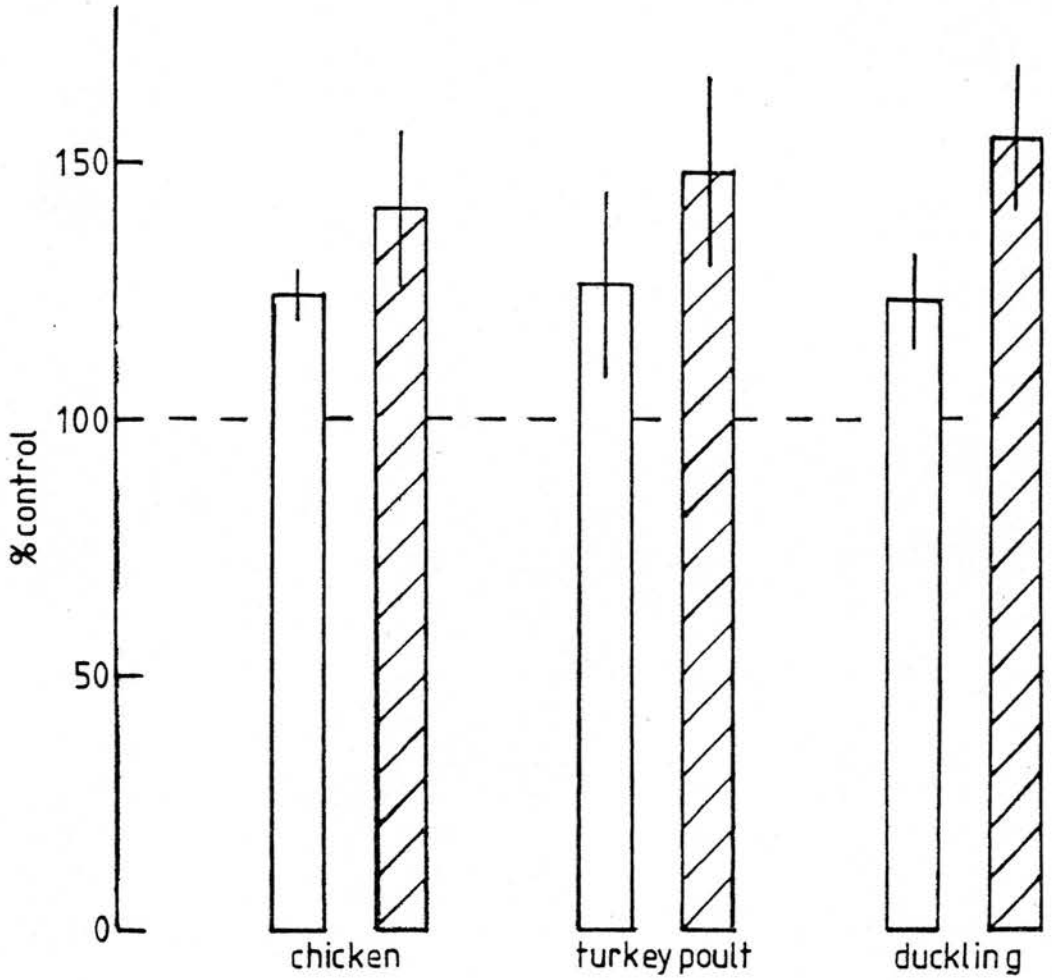


Figure 24. The influence of furazolidone (0.04% w/w, 10 days) on the concentrations of lactate (open columns) and pyruvate (cross-hatched columns) in the blood of chickens, turkey poults and ducklings. Ordinate : the concentrations of lactate or pyruvate expressed as % control. Each column represents a mean value obtained from 5-6 birds, the s.e.m. being depicted by vertical bars. In each species the concentrations of lactate and pyruvate were significantly increased following furazolidone treatment ($P < 0.05 - < 0.001$).

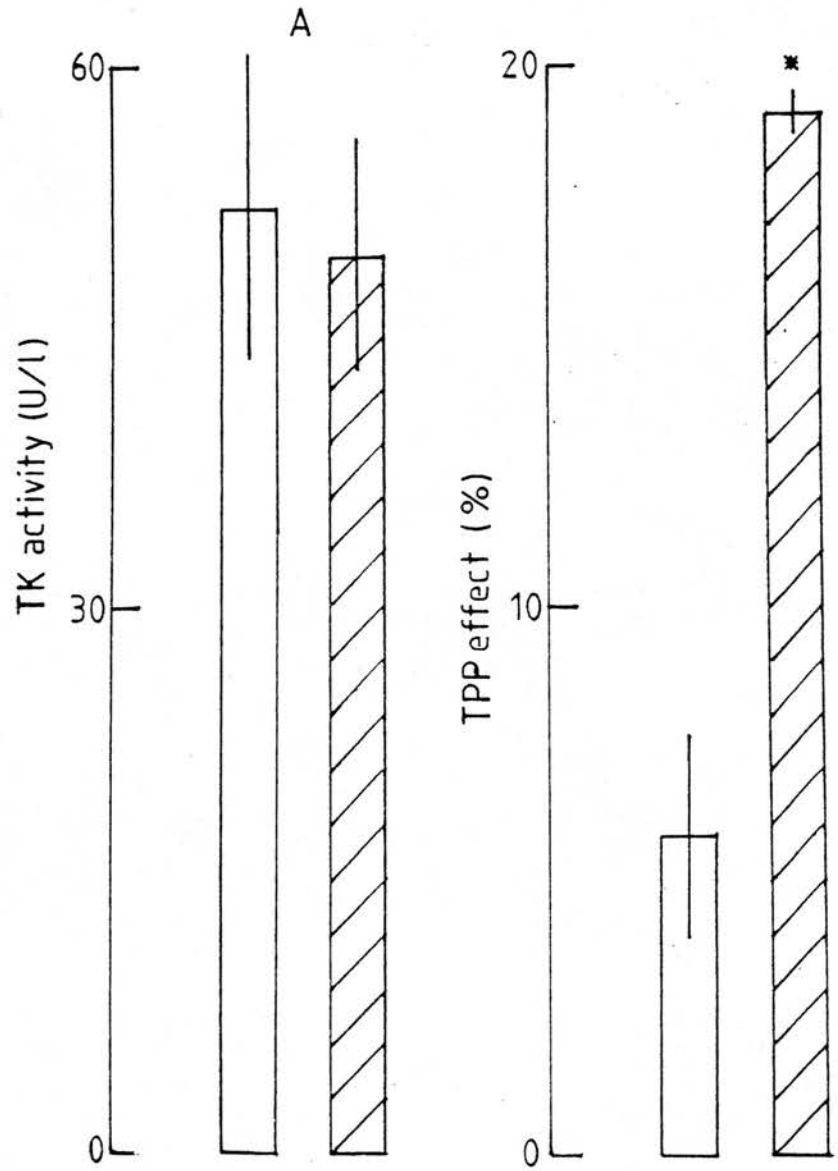


Figure 25. Effect of furazolidone (0.04% w/w, 10 days) on the activity of transketolase (TK) and the thiamin pyrophosphate (TPP) effect in chicken haemolysate. Left ordinate : TK activity (u/l). Right ordinate : TPP effect expressed as % increase in TK activity upon addition of TPP. The columns represent the mean values obtained from 5 control (open) and furazolidone-treated chickens (cross-hatched). The vertical bar at the head of each column depicts the s.e.m. The asterisk marks a significant increase in the TPP effect in the furazolidone-treated chickens ($P < 0.02$).

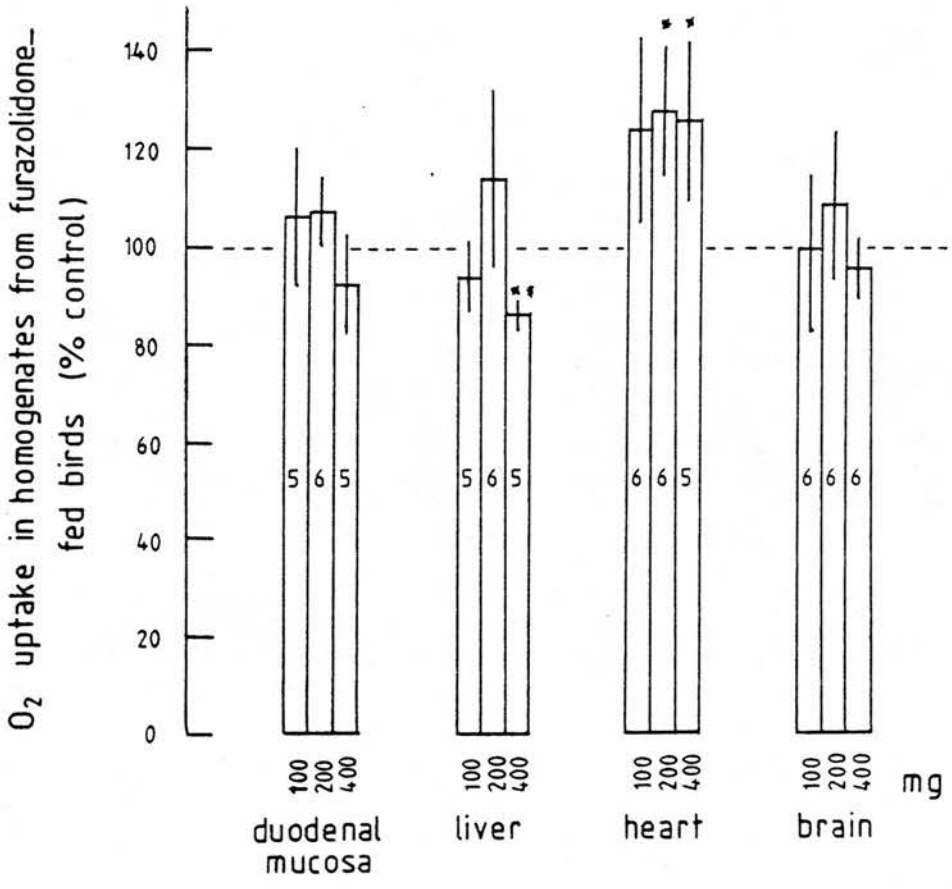


Figure 26. Effect of furazolidone (0.04% w/w, 10 days) on the oxygen consumption of chicken organs.
Ordinate : oxygen consumption of homogenized tissue expressed as a percentage of the control values. The amounts of tissue (mg duodenal mucosa, liver, heart or brain) in the incubates is shown on the abscissa. Each column represents a mean value obtained from 5 or 6 chickens, and the vertical bar at the head of the columns depicts the standard error of the means. Furazolidone increased the oxygen consumption of heart (200 or 400 mg, $P < 0.02$) and reduced that of liver (400 mg, $P < 0.001$).

Acknowledgements

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INHIBITION OF MONOAMINE OXIDASE BY FURAZOLIDONE IN THE CHICKEN AND THE INFLUENCE OF THE ALIMENTARY FLORA THEREON

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1 The addition of furazolidone to the feed at the therapeutic level (0.04% w/w, 10 days) inhibited monoamine oxidase (MAO) activity by 47 to 72% in chicken duodenal mucosa, heart and brain, but in the liver the enzyme activity was unaffected by the treatment.

2 Furazolidone (200 mg/kg) administered by crop tube inhibited MAO activities in duodenal mucosa, liver, heart and brain.

3 Furazolidone (200 mg/kg) injected intramuscularly did not inhibit MAO activity in the chicken.

4 Pretreatment of the chickens with intramuscular neomycin did not antagonize the inhibition of MAO activity produced by furazolidone (200 mg/kg, crop tube).

5 Pretreatment with neomycin by crop tube to suppress the alimentary flora significantly reduced the effect of furazolidone on MAO activity, suggesting that the drug was transformed by the alimentary flora to an active metabolite which subsequently inhibited MAO activity in other organs.

6 Furazolidone in the feed (0.04% w/w, 10 days) or administered by crop tube (200 mg/kg) had no effect on the activity of aminopyrine demethylase in chicken liver.

7 The activity of aspartate transaminase in plasma was unaffected by the addition of furazolidone to the feed (0.04% w/w, 10 days).

Introduction

Furazolidone [(*N*-5-nitro-2-furfurylidene)3-amino-2-oxazolidine] is widely used in the prevention and treatment of certain bacterial and protozoal diseases which affect poultry (Harwood & Stunz, 1954; Rogers, Belloff, Paul, Yurchenco & Gever, 1956). At slightly above the therapeutic level the drug inhibits growth, produces anaemia and arrests spermatogenesis in the chicken (Cooper & Skulski, 1956; MacDonald & Beilharz, 1961; Ehlhardt, Beuving & Haye, 1975) and may also produce cardiac dilatation and ascites (Feron & Van Stratum, 1966). The biochemical lesion associated with these toxic signs has not been defined.

In rat and man, furazolidone inhibits monoamine oxidase (MAO, monoamine:O_2 oxidoreductase (deaminating) EC 1.4.3.4.). However, the drug does not inhibit MAO *in vitro* suggesting that a transformation product, possibly 2-diethylhydrazine, is responsible for the enzyme inhibition *in vivo* (Stern, Hollifield, Wilk & Buzard, 1967; Pettinger, Soyangco & Oates, 1968).

In the present experiments we have investigated the effect of furazolidone on the activity of MAO in the chicken. A preliminary account of some of the work was given to the British Pharmacological Society (Ali & Bartlet, 1979).

Methods

Animals

Thornber chickens (8 weeks old, either sex) were obtained from the Poultry Research Centre, Kings Buildings, Edinburgh. The birds were fed a layer mash (Douglas of Dalkeith, Edinburgh) which was free from growth promoting and anticoccidial drugs. In some experiments furazolidone was administered in the feed at the therapeutic level of 0.04% w/w for 10 days, the controls receiving unmedicated feed. In other experiments the drug (200 mg/kg) was administered by intramuscular injection or crop tube, control birds receiving 0.9% w/v aqueous NaCl or aqueous acacia (20% w/v), respectively.

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Preparation of tissues

Chickens were killed by decapitation and the tissues rapidly removed and chilled on ice. The lumen of the duodenum was washed with chilled saline before cutting it open and scraping away the mucosa with a glass slide. The heart was opened and blotted to remove blood. The tissues were cut into small pieces and homogenized with six strokes in a Teflon homogenizer in an ice bath. Homogenates (20% w/v) of each organ in 0.5 M phosphate buffer (pH 7.4) were prepared for the estimation of MAO activity. For the estimation of aminopyrine demethylase activity, a liver homogenate (25% w/v) in isotonic KCl (1.15% w/v) was centrifuged at 10,000 *g* for 20 min in an MSE High Speed 18 Refrigerated centrifuge at 5°C, and the microsome-rich supernatant used.

Measurement of enzyme activity

Monoamine oxidase activity MAO activity was estimated according to the method of Krajl (1965), in which the production of 4-hydroxyquinoline from the oxidative deamination of kynuramine is measured. The incubation mixture contained kynuramine dihydrobromide (100 μg), 0.5 M phosphate buffer pH 7.4 (0.5 ml), homogenate (equivalent to 0.78 mg duodenal mucosa, 3.13 mg liver or brain or 12.5 mg heart) and de-ionised water to a final volume of 3 ml. An incubate without substrate served as the blank. The reaction mixtures were incubated at 37°C for 30 min under air and with shaking. The incubates were then deproteinised with perchloric acid (0.6 M) as recommended by Century & Rupp (1968) followed by centrifugation at 900 *g* for 10 min at 5°C. Supernatant (1 ml) was mixed with 1 N NaOH (2 ml) and placed in a quartz cuvette. The solution was activated at 315 nm and the peak in the fluorescence scan at 380 nm measured in an Aminco-Bowman spectrofluorimeter. The presence of substances affecting the fluorescence of 4-hydroxyquinoline was sought by mixing equal volumes of tissue blank and 4-hydroxyquinoline solution. In all cases the fluorescence of the mixture was half that of the undiluted solution of 4-hydroxyquinoline, demonstrating an absence of augmentation or quenching of fluorescence.

Aminopyrine demethylase activity was estimated in the 10,000 *g* supernatant fraction of liver. The conditions of the incubation were those described by Mazel (1971), except that the concentrations of aminopyrine and semicarbazide in the incubation mixture were both 10 mM. The formaldehyde produced in the reaction was estimated by the method of Nash (1953).

Aspartate transaminase [AST (EC 2.6.1.1.)] activity was estimated in chicken plasma by the method described by Wilkinson, Baron, Moss & Walker (1972). This measures the rate at which absorbance at 340 nm decreases as NADH is oxidized to NAD. Blood was withdrawn from a wing vein by means of a heparinised syringe fitted with a 21G needle (external diameter 0.8 mm) and spun down in a refrigerated centrifuge (5°C) at 900 *g* for 20 min to separate plasma.

Statistical analysis

The values in the text, tables and figures are means ± the standard error of the mean (± s.e. mean) with the number of observations in parentheses. Significant difference between means was estimated by the *t* test and probability (*P*) values are given. A value of $P < 0.05$ is regarded as significant.

Drugs and chemicals

A premix containing 4% w/w furazolidone (Neftin) was used for the addition of the drug to the feed, and a micronized preparation of furazolidone (particle size 5 μm) for administration by injection and crop tube. Acetylacetone (Koch Light), aminopyrine (4-dimethylamine-antipyrine 97%) (Ralph Emanuel), L-aspartic acid (Sigma), D-glucose-6-phosphate monosodium salt (Sigma), 4-hydroxyquinoline (Sigma), kynuramine dihydrobromide (Sigma), malate dehydrogenase (Sigma), neomycin sulphate (70% soluble powder, veterinary) (Squibb & Sons), nicotinamide (Sigma), nicotinamide adenine dinucleotide phosphate (Sigma), nicotinamide adenine dinucleotide, reduced form (Sigma) and 2-oxoglutaric acid (Sigma) were used. The other chemicals were Analytical Reagent grade.

Results

In the chicken the activity of MAO in heart and liver is reported to increase with age (Ignarro & Shideman, 1968), and our data (Table 1) confirmed this trend.

Table 1 Monoamine oxidase (MAO) activity in homogenates of organs from chickens of different ages

	MAO activity (*4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1}*)		
	1 day	*8 weeks*	P
Duodenum	36.9 ± 5.1 (6)	90.7 ± 12.5 (8)	<0.02
Liver	2.5 ± 0.4 (6)	22.3 ± 1.9 (7)	<0.001
Heart	1.2 ± 0.1 (6)	3.3 ± 0.3 (7)	<0.001
Brain	12.7 ± 1.1 (7)	15.6 ± 2.0 (7)	>0.2

The values in the table are means ± s.e. means (No. of animals).

Thus in our other experiments all the birds were killed at the same age, 8 weeks, at which age the activities of MAO in control organs was consistent.

Effect of furazolidone in the feed on monoamine oxidase activity

When furazolidone was added to the feed at a therapeutic concentration of 0.04% w/w for 10 days, the activity of MAO was reduced significantly in duodenal mucosa, heart and brain, and not in liver (Table 2). Another experiment investigated the recovery in enzyme activity after discontinuing the medicated feed. This experiment confirmed the effect of feeding furazolidone on MAO activity, which was inhibited in the duodenal mucosa ($P < 0.001$), heart ($P < 0.02$) and brain ($P < 0.02$), and not in liver ($P > 0.1$) (Figure 1). Seven days after discontinuing furazolidone in the feed a significant recovery of MAO activity was only evident in the duodenal mucosa ($P < 0.02$). Fourteen days after discontinuing the drug the recovery of MAO activity was complete in each organ, there being no significant difference from the control values ($P > 0.1$ for each organ).

Effect of furazolidone by crop tube or intramuscular injection on MAO activity

The drug was administered at a dose of 200 mg/kg body weight, which is about half the reported LD_{50} in the chicken (Radchuk, 1965). Furazolidone administered by crop tube inhibited MAO activity in the liver as well as that in duodenal mucosa, heart and brain (Table 3). The time course of the inhibition was different in various organs, however, being maximal in the liver 12 h after drug administration and in the duodenal mucosa and brain after 24 h.

In another experiment furazolidone (200 mg/kg) was administered by crop tube or intramuscular injection and MAO activities estimated 24 h later. This experiment confirmed that the drug given by crop tube inhibited MAO activity in the liver as well as in the other organs studied ($P < 0.001$ for each organ, 6 birds). In contrast, furazolidone injected intramuscularly did not affect MAO activity in any of the organs studied ($P > 0.1$ for each organ, 6 birds).

Effect of neomycin on the inhibition of monoamine oxidase by furazolidone

In this experiment the birds were given neomycin, either 20 mg by intramuscular injection or 200 mg by crop tube twice daily for 5 days before MAO estimation. Furazolidone (200 mg/kg) was administered by crop tube to saline- and neomycin-treated birds 24 h before the enzyme estimation. Neomycin did not affect MAO activity and the values obtained from chickens pretreated with saline or neomycin and given acacia mucilage (crop tube) were pooled and used as controls. As shown in Table 4, furazolidone administered by crop tube inhibited MAO activity in each organ as before ($P < 0.01$), and pretreatment

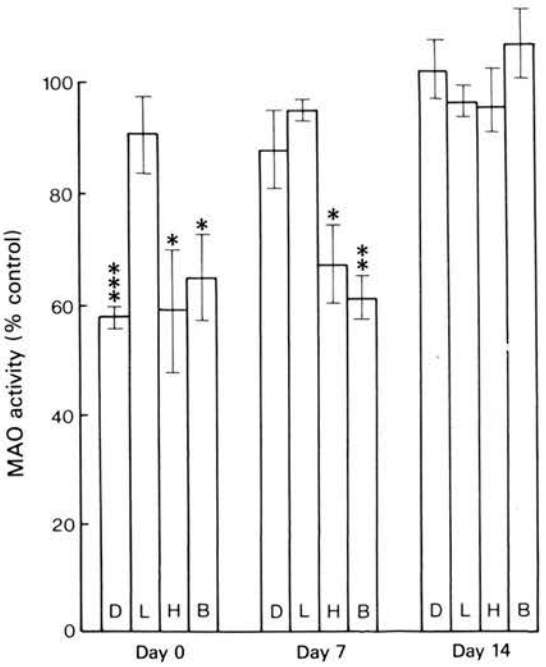

Figure 1 Monoamine oxidase activity (MAO) in homogenates of chicken organs after discontinuing furazolidone in the feed (0.04% w/w, 10 days). Ordinate scale: MAO activity as a percentage of the control value. The columns represent mean values for duodenal mucosa (D), liver (L), heart (H) and brain (B) at 0, 7 and 14 days after discontinuing furazolidone medication. The vertical bar at the head of each column depicts s.e. mean (3 birds). The asterisks mark significant inhibition of MAO, at the 2% (*), 0.2% (**) and 0.1% (***) levels.

Table 2 Effect of feeding furazolidone (0.04% w/w, 10 days) on monoamine oxidase (MAO) activity in homogenates of chicken tissue

	MAO activity (4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1})		
	Control	*Furazolidone*	P
Duodenum	92.6 ± 10.0 (10)	58.9 ± 7.5 (9)	<0.02
Liver	21.9 ± 3.9 (8)	22.3 ± 1.9 (8)	>0.9
Heart	3.4 ± 0.3 (7)	1.7 ± 0.3 (6)	<0.002
Brain	15.5 ± 2.0 (7)	9.4 ± 0.7 (6)	<0.02

The values in the table are means ± s.e. means (No. of animals).

with neomycin by the intramuscular route did not affect the MAO inhibition ($P > 0.1$). On the other hand, pretreatment with neomycin by crop tube significantly reduced the MAO inhibiting action of furazolidone in each organ ($P < 0.01$ to < 0.001). Following oral administration of neomycin and furazolidone the activity of MAO was less than the control value in the liver ($P < 0.002$) and brain ($P < 0.05$) but not in the duodenal mucosa ($P > 0.1$) and heart ($P > 0.05$). Thus the suppression of the MAO inhibiting action of furazolidone by the oral neomycin was complete in the duodenal mucosa and heart and not in the liver and brain.

Effect of furazolidone on aspartate transaminase and aminopyrine demethylase activities

Addition of furazolidone (0.04% w/w, 10 days) to the feed had no significant effect on the activity of aspartate transaminase in plasma (Table 5).

Table 3 Effect of time on monoamine oxidase (MAO) inhibition produced by furazolidone (200 mg/kg) given by crop tube

	MAO activity (*4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1}*)			
Time (h) after furazolidone	*Duodenal mucosa*	*Liver*	*Heart*	*Brain*
0	95.95 ± 6.21 (3)	29.72 ± 4.70 (3)	2.51 ± 0.21 (3)	15.41 ± 1.76 (3)
12	40.43 ± 0.92 (3)	9.93 ± 0.31 (3)	0.90 ± 0.01 (3)	8.80 ± 0.41 (3)
24	34.66 ± 1.67 (3)	11.65 ± 0.53 (3)	0.92 ± 0.10 (3)	7.02 ± 0.85 (3)
48	36.63 ± 2.65 (3)	15.27 ± 1.12 (3)	0.82 ± 0.06 (3)	8.04 ± 0.18 (3)

The values in the table are means ± s.e. means (No. of animals).

Table 4 Monoamine oxidase (MAO) activity in homogenates of chicken organs 24 h after administration of furazolidone (200 mg/kg, crop tube) and the influence of neomycin thereon

	MAO activity (*4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1}*)				P *Intramuscular neomycin* [*cf.(3) and (2)*]	P *Oral neomycin* [*cf. (4) and (2)*]
Duodenal	*(1) Control*	*(2) Furazolidone*	*(3) Furazolidone + *neomycin (i.m.)*	*(4) Furazolidone + **neomycin (oral)*		
mucosa	93.71 ± 5.29 (15)	32.83 ± 6.65 (6)	38.10 ± 2.20 (3)	75.19 ± 11.54 (6)	>0.1	<0.01
Liver	24.88 ± 1.90 (15)	8.95 ± 1.26 (6)	9.69 ± 0.25 (3)	15.15 ± 1.38 (6)	>0.1	<0.01
Heart	2.66 ± 0.13 (15)	0.81 ± 0.09 (6)	1.18 ± 0.30 (3)	2.32 ± 0.14 (6)	>0.1	<0.001
Brain	11.77 ± 0.71 (15)	7.11 ± 0.65 (6)	7.38 ± 0.37 (3)	10.05 ± 0.27 (6)	>0.1	<0.002

The values in the table are means ± s.e. means (No. of animals).
* Neomycin, 20 mg/bird by intramuscular injection, twice daily for 5 days before the MAO estimation.
** Neomycin, 200 mg/bird by crop tube, twice daily for 5 days before the MAO estimation.

Table 5 Effect of furazolidone on the activities of aminopyrine demethylase in liver and aspartate transaminase in plasma from chickens

Expt	*Treatment*	*Aminopyrine demethylase* (*H.CHO μmol* ml^{-1} h^{-1}*)	*Aspartate transaminase* (*iu/l*)
1	Control feed	0.37 ± 0.04 (6)	56.2 ± 5.2 (5)
	Furazolidone, 0.04% w/w in feed, 10 days	0.37 ± 0.05 (6)	59.1 ± 5.2 (8)
2	Acacia mucilage (crop tube)	0.30 ± 0.03 (5)	
	Furazolidone, 200 mg/kg (crop tube)	0.35 ± 0.08 (6)	

* Per ml of 10,000 *g* supernatant of liver homogenate (25% w/v).
The values in the table are means ± s.e. means (No. of animals).
The drug had no significant effect on the activities of either enzyme.

In two experiments the effect of furazolidone on aminopyrine demethylase activity in a microsome-rich supernatant fraction of chicken liver was investigated (Table 5). In one experiment the drug was administered in the feed at the therapeutic dose, and in the other by crop tube at a dose of 200 mg/kg 24 h before estimation of the enzyme activity. In both experiments the activity of aminopyrine demethylase was unaffected by the drug.

Discussion

Furazolidone administered in the feed at the therapeutic dose inhibited MAO activity in the duodenal mucosa, heart and brain, but in the liver the enzyme activity was unaffected by the treatment. MAO is inhibited by a transformation product of furazolidone (Stern *et al.*, 1967; Pettinger *et al.*, 1968), and MAO inhibition would be expected at the site of transformation. As MAO activity was not inhibited in the liver, it seems improbable that the transformation of furazolidone occurred here. Following withdrawal of medication, the enzyme activity recovered more promptly in the duodenal mucosa than in the other organs. The recovery of activity was probably due to synthesis of new enzyme, as dialysis does not restore activity to MAO inhibited by furazolidone (Stern *et al.*, 1967).

Furazolidone administered by crop tube inhibited the activity of MAO in the duodenal mucosa, liver, heart and brain. Thus furazolidone given as a bolus inhibited the hepatic enzyme although it did not do so when fed continuously. In the hepatocytes, the furazolidone metabolite may inhibit mitochondrial MAO, or, it may be metabolized further and/or excreted with bile. The present observations suggest that clearance of the metabolite was insufficient to protect hepatic MAO from inhibition when the drug was given as a bolus (200 mg/kg), but was sufficient when it was given as a feed additive (0.04% w/w, 10 days). Following furazolidone by crop tube a maximum inhibition of MAO in the liver was found after 12 h and in other organs after 24 h. This observation suggests that hepatic clearance of the furazolidone metabolite resulted in a briefer inhibition of MAO in the liver than elsewhere.

Furazolidone administered by intramuscular injection did not affect the activity of MAO in any of the organs studied. This observation suggests that the conversion of the drug to an MAO inhibitor did not occur in chicken tissues. However, this finding may not extend to other species as furazolidone given by intravenous or intraperitoneal injection is reported to inhibit MAO in the rat (Stern *et al.*, 1967).

The alimentary flora might have produced the metabolite which inhibited MAO activity. To test this hypothesis furazolidone was given by crop tube after suppressing the alimentary flora with neomycin. Pretreatment with neomycin by crop tube antagonized the action of furazolidone on MAO activity, but neomycin injected intramuscularly at 1/10th of the oral dose did not do so. Less than 10% of neomycin is absorbed from the alimentary tract (Waksman, 1953; Freyburger & Johnson, 1956), so in the present experiments absorbed neomycin could not have influenced the action of furazolidone on MAO activity. The inhibition of MAO activity by furazolidone was suppressed by unabsorbed neomycin, which supports the hypothesis that the alimentary flora transforms furazolidone to an active metabolite which is subsequently responsible for the inhibition of MAO activities in other organs.

Inclusion of furazolidone in the feed (0.04% w/w, 10 days) did not affect the activity of aspartate transaminase in plasma. This is a cytoplasmic enzyme, and the present observation suggests that furazolidone did not produce a detectable damage in cell membranes. However, furazolidone at a higher dose (0.05% w/w, 28 days) has been reported to increase significantly the activity of this enzyme in turkey plasma (Staley, Noren, Bandt & Sharp, 1978).

Many drugs inhibit both MAO and mixed function oxidase (Kato, Takanaka & Shoji, 1969). Aminopyrine demethylase activity in chicken liver, however, was unaffected by furazolidone treatment (0.04% w/w, 10 days, or 200 mg/kg by crop tube). Thus furazolidone at the recommended therapeutic dose would not be expected to potentiate the actions of other drugs metabolized by mixed function oxidase.

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Inhibition of monoamine oxidase by furazolidone in the chicken: influence of the alimentary flora

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Furazolidone is widely used in the prevention and treatment of certain bacterial and protozoal diseases which affect poultry. Oral administration of the drug in rat and man has been shown to inhibit monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating) EC 1.4.3.4.]. Furazolidone does not inhibit MAO *in vitro*, however, suggesting that a transformation product is responsible for the enzyme inhibition *in vivo* (Stern, Hollifield, Wilk & Buzard, 1967; Pettinger, Soyangco & Oates, 1968).

[British Pharmacopoeia (Veterinary), 1977] inhibited MAO activity by 50% in heart, 39% in brain, 36% in duodenal mucosa and not in liver. The inhibition of the enzyme in heart, brain and duodenal mucosa were all significant at the 2% level.

Furazolidone (200 mg/kg) injected intramuscularly did not affect MAO activity, but administered by crop tube it inhibited the enzyme by 47–72% (Table 1). These observations suggest that the transformation of furazolidone to a MAO inhibitor occurred in the gut. Pretreatment with neomycin to suppress the alimentary flora reduced the effect of furazolidone on MAO activity (Table 1). Thus in the chicken MAO inhibition by furazolidone seems to depend on the presence of the alimentary flora.

B.H.A. is a British Council student. We thank Eaton Laboratories and Squibb Ltd. for gifts of furazolidone and neomycin respectively and the Wellcome Unit for housing the birds.

Table 1 Monoamine oxidase activity in homogenates of chicken organs 24 h after administration of furazolidone, and the influence of neomycin thereon

Expt.	Drugs	*Monoamine oxidase activity (μmol 4-hydroxyquinoline/g/h)* Duodenal mucosa	Liver	Heart	Brain
1	Saline, i.m.	71.00 ± 3.29 (6)	20.50 ± 1.46 (6)	2.06 ± 0.22 (6)	7.97 ± 0.41 (6)
	Furazolidone (200 mg/kg), i.m.	73.02 ± 4.61 (6)	20.51 ± 1.74 (6)	2.02 ± 0.20 (6)	7.72 ± 0.52 (6)
2	Acacia mucilage, orally	104.60 ± 8.77 (6)	27.67 ± 3.80 (6)	2.94 ± 0.22 (6)	13.42 ± 0.41 (6)
	Furazolidone (200 mg/kg), orally	32.83 ± 6.65 (6)**	8.95 ± 1.26 (6)**	0.81 ± 0.09 (6)**	7.11 ± 0.65 (6)**
3	Neomycin (200 mg/bird, twice daily for 5 days), orally	85.33 ± 8.17 (6)	22.52 ± 2.91 (6)	2.71 ± 0.19 (6)	10.50 ± 0.59 (6)
	Neomycin (as above) + furazolidone (200 mg/kg), orally with the last dose of neomycin	75.19 ± 11.54 (6)	15.15 ± 1.38 (6)**	2.32 ± 0.14 (6)*	10.05 ± 0.27 (6)

The values in the table are means ± s.e. means (No. of observations).
* $P < 0.01$. ** $P < 0.001$.

We have investigated the effect of furazolidone on MAO activity in the chicken. The birds (Thornbers, 8 weeks old) were killed at the end of a feed trial or 24 h after administration of the drug by intramuscular injection or crop tube. Selected organs were homogenized for the estimation of MAO activity by the method of Kramal (1965), in which formation of 4-hydroxy-quinoline from kynuramine is measured spectrofluorometrically.

The addition of furazolidone to the feed at the therapeutic concentration of 0.04% w/w for 10 days

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Some actions of furazolidone in poultry

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Furazolidone has recently been shown to inhibit monoamine oxidase (MAO) activity in the chicken (Ali & Bartlet, 1980), and, in the present experiments, in turkey poults and ducklings. When administered by crop tube furazolidone was about equipotent in inhibiting MAO activity in these three species (Figure 1).

Inclusion of furazolidone in the feed at a concentration of 0.04% w/w for 10 days produced anorexia in chickens, turkey poults and ducklings, but no mortality. The treatment produced a similar degree of MAO inhibition in the hearts, brains and duodenal mucosae from the three species, enzymatic activity being inhibited by 29 to 59% ($P < 0.05$ to < 0.001). Only in the duckling was a significant inhibition of MAO activity found in the liver ($P < 0.01$). Furzolidone 0.04% w/w for 10 days is the therapeutic treatment for galliformes (Veterinary Data Sheet, 1980), the recommended dose for ducks being 0.01% w/w of the drug for 7 days.

Monoamines extracted from brain with perchloric acid were purified on a cation exchange resin (Amberlite CG-50, ammonium form). 5-Hydroxytryptamine (5-HT) was estimated by its fluorescence in 3 M HCl and catecholamines by the trihydroxyindole method. The concentration of 5-HT in brains of ducklings fed furazolidone (0.04% w/w for 10 days) was 0.57 ± 0.06 μg/g tissue (mean ± s.e., 5 birds), and in paired controls 0.39 ± 0.04 μg/g. This increase in brain 5-HT ($P < 0.05$) was selective, as concentrations of adrenaline and noradrenaline in the brain were unchanged after feeding furazolidone to the ducklings ($P > 0.1$).

The pressor actions of graded doses of tyramine and noradrenaline were measured in chickens anaesthetized with phenobarbitone sodium (180 mg/kg, i.v.). Responses to tyramine in preparations made from chickens fed furazolidone (0.04% w/w for 10 days) were about 70% greater than those in control preparations. The pressor action of noradrenaline was unaffected by the treatment.

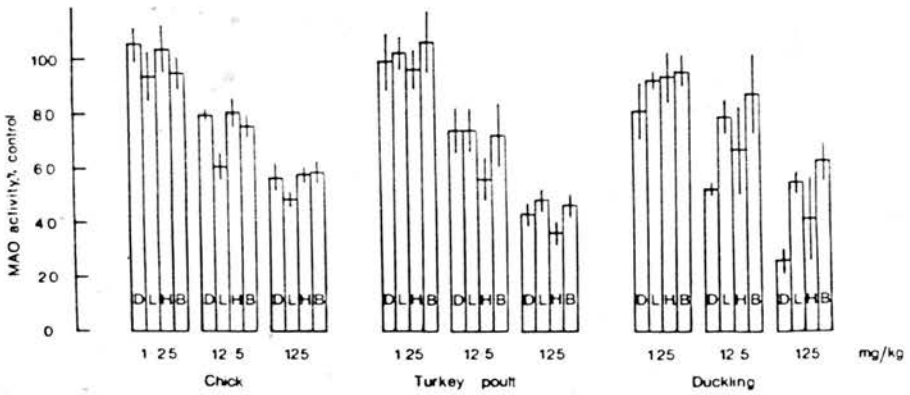

Figure 1 The inhibition of monoamine oxidase (MAO) activity in chicks, turkey poults and ducklings 24 h after furazolidone administered by crop tube. Ordinate: MAO activity estimated by the method of Krajl (1965), expressed as a percentage of the value in control birds. Abscissa: doses of furazolidone (mg/kg) administered to birds aged 2 to 12 days. The columns represent mean values for duodenal mucosa (D), liver (L), heart (H) or brain (B) from 5 chicks, 5 turkey poults and 3 ducklings; the vertical bars at the head of the columns represent the s.e. Furazolidone inhibited MAO activity in the tissues of the three species to about the same extent, except that in the heart it was greater in turkey poults than in chicks ($P < 0.05$) and in duodenal mucosa more marked in ducklings than in chicks or turkey poults ($P < 0.05$).

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